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CALCIFICATION OF THE BREAST FOLLOWING A TYPHOID ABSCESS.[1]

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AND

H. H. HAZEN, M.D.

PLATE I.

The general interest, both pathological and clinical, in the processes of calcification in the animal œconomy, as well as the increasing frequency of the therapeutical use of various salts of calcium, justify a consideration of the following remarkable case.

D. I., a colored house maid, aged 16, was admitted to the Johns Hopkins Hospital on October 2, 1905, on the ninth day of a severe typhoid fever. Her family and personal history were unimportant. Eight days before entry she began to complain of headache and had a chill. On the succeeding day there was abdominal discomfort and occasional "pains in the bones." She felt too ill to work and took some medicine which was followed by diarrhœa—from two to four fluid movements a day—which continued up to the date of entry. There was complete anorexia, and, on several occasions, nausea and vomiting.

On admission the patient was dull and apathetic. The tongue was heavily coated; the pulse, 132; temperature, 101.8°. There were a few coarse râles at the left base. The abdomen was moderately distended and tympanitic. The spleen was not palpable, although the dulness appeared to be increased. Examination of the blood showed: Red blood corpuscles, 3,924,000; colorless corpuscles, 6000; hæmoglobin, 77 per cent. The urine showed a trace of albumin and a positive Diazo reaction. The blood pressure (Riva-Rocci, broad band) was 105. Treatment: milk and albumen water, 120 c.c. alternating every two hours; tub baths, at 85° F., every three hours if the temperature be above 102.5°. On the following day the Widal reaction was positive. The patient soon became very ill. There was considerable tympanities at first which was relieved by turpentine stoops. On October 8 the pulse was slightly irregular; blood pressure (Riva-Rocci, broad band) between 80 and 90. The temperature of the baths was reduced to 75°, and strychnine 0.001 (grs. 1/60) was ordered every four hours, as well as whiskey 15 c.c. (℥ss) p. r. n. The leucocytes varied from 4000 to 7000 to the cubic millimetre.

On October 9, the sixteenth day of the disease, there was a semi-fluid stool with a trace of blood.

[1] Read before the American Association of Pathologists and Bacteriologists, at Baltimore, 19 May, 1906.

On October 12, the nineteenth day, about 340 c.c. of blood were passed in three stools. The temperature fell from 102.7° in the morning to 99.4° at noon. The pulse rose to 164, the pressure falling at one time to 81.5. At 11 P. M. an infusion of 500 c.c. of 1 per cent. solution of calcium chloride was given under the left breast. The hæmoglobin dropped to 50 per cent. in the evening. The coagulation time was five minutes. An ice bag was placed upon the abdomen; the baths were omitted, and lactate of calcium 2.0 (gr. XXX) every four hours was prescribed.

On the following day, the coagulation time was eleven minutes. Ice sponges were begun again. On October 15, the twenty-second day of the disease, 150 c.c. of blood appeared again in several stools but without any essential change in the condition of the patient. The coagulation time was four minutes on the day preceding the hæmorrhage, and eight and a half minutes on the day following. On October 18, the twenty-fourth day, there was again blood in the stools, for the most part in the shape of old clots, amounting to from 200–800 c.c. by measure. On the 19th, the red blood corpuscles had fallen to 2,200,000; the colorless corpuscles to 3100. From this time on, there was a steady, gradual improvement. On October 23, the thirty-fifth day of the disease, the lactate of calcium was omitted. On the same day, a large abscess was discovered involving the lower half of the left breast, and dissecting around in the interspace just below this as far as the mid-axillary line. There was definite fluctuation just beneath the skin, and harder masses, 1 to 2 cm. in length, could be felt floating free in the pus. The breast was little deformed, the dissection occuring chiefly in the areolar tissue. The point of insertion of the needle with which the infusion of calcium chloride was made fifteen days previously, was not affected, but the abscess was just above this spot, and evidently involved a part of the area in which the infusion was made. The abscess was on the point of rupture and, indeed, did rupture before the operation. Under cocaine, several long incisions were made, two over the abscess proper and one in its extension in the mid-axillary line. A quantity of thin brown pus containing several large sloughs was evacuated. The wound was packed with gauze for drainage.

On October 31, three days later, the wound was irrigated with boric acid and dressed. There was still some sloughing of the actual breast tissues and a prolongation of the abscess up, over and under the mammary gland. On the whole the wound looked fairly clean; it was packed with iodoform gauze. Cultures taken at the time of the opening of the abscess showed Staphylococcus aureus and Bacillus typhosus.

On November 8, eight days later, an examination of the wound, which had been left open with an iodoform dressing, revealed a cartilaginoid induration about the upper part of the opening. There had been an extension around toward the back from the lower part of the drainage incision. On the whole, the wound was clean and of good color. The patient steadily improved, the temperature reaching the normal point on the tenth of November. A light general diet had been allowed for the preceding week.

On November 16, it was noted that the denuded tissue in the wound was slowly changing into a necrotic and almost stony mass. Along the outer side of the breast there was a chain of glands, somewhat swollen and extremely hard. Neither glands nor wound were painful. The suppuration had not extended further toward the back, and the edge of the wound was closing in.

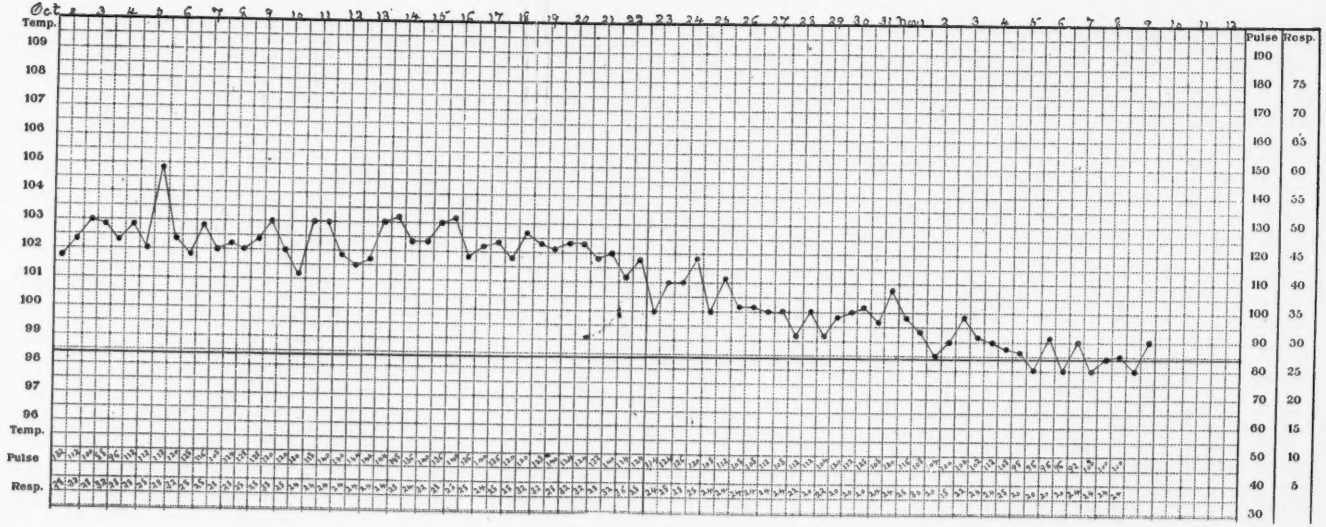

FIG. 1.—Temperature Chart.

On November 20, the following note was made (W. S. T.): "About the opening of the wound there is a perfectly distinct rim of stony hardness. The stony rim of the crater-like opening extends back on the median side about 2 cm. from the edge; on the outer side it is less extensive. The granulations protruding from the outer part of the wound are rather pale, and in them are to be seen whitish streaks and patches that look altogether like the guanin deposits in old ham. These areas feel hard and stony. Passing into the cavity of the wound, the under surface of the protruding granulations corresponds exactly in consistency to that of the outer rim of the crater, the forceps striking similar calcareous areas. In the outer part of the breast, above the opening, and extending along the second interspace are a number of other hard, nodular masses. The largest of these, which is situated a little anteriorly to the mid-axilla, in the third interspace, is as large as a good-sized hickory nut. Above and to the mesial side of this, the nodules are smaller. A number of smaller, indurated areas are to be felt throughout the left breast."

Bits of the white, gritty deposit were removed from the edges of the wound by forceps. These masses were separated easily and with but little bleeding. On burning they emitted a brilliant white light. They dissolved in hydrochloric acid without effervescence. By quantitative analysis[1] it was determined that the calcium, estimated as CaO, comprised 20 per cent. of the dry mass. Curettings of the tissue were examined for tubercle bacilli without result. A guinea-pig, inoculated with scrapings from the edge of the wound, and killed several months later, was free from tuberculosis. Bits of tissue from the wall of the abscess were sent to Dr. Bloodgood on November 14, the following note being made: "The specimens consist of bits of substance that are very firm and seen to be composed of fibrous tissue with a number of small areas of calcification. The section shows granulation tissue on a pretty fibrous basis. In the granulation tissue there are a good many polynuclear leucocytes; lymphoid cells predominate. There are some epithelioid cells. No giant cells or tubercles. Areas of calcification are seen." Further examination showed occasional characteristic giant cells.

The tissue was also examined by Dr. Bunting who made the following note: "The specimen consists of granulation tissue upon a basis of white fibrous tissue. The surface of the granulation tissue shows a fibrin network containing clotted blood, and the immediately underlying portion of the tissue is invaded by polymorphonuclear leucocytes. The granulation tissue is apparently firm and fibrous although, toward the surface, it is extremely cellular, the vessels being surrounded by large numbers of mononuclear cells, both large and small and of the plasma cell variety. Among these one mitotic figure is found. In the deeper layer of the granulation tissue the fibroblasts predominate. In this portion of the tissue there are numerous areas which take a diffuse light bluish stain with hæmatoxylin which on high power seem slightly granular. The tissues in these areas are somewhat swollen and almost hyaline in appearance. The nuclei are pyknotic and the areas are somewhat surrounded and invaded by polymorpho-

[1] The mass was dissolved in hydrochloric acid, the subsequent procedure being that advised by Neubauer and Vogel for the estimation of calcium in the urine. Analyse des Harns, Wiesbaden, 1898, 746.

nuclear leucocytes. In the deeper layers there are numerous multinuclear foreign body giant cells which do not seem to bear any definite relation to the bluish areas described."

The accompanying photograph (Plate I), taken between November 20 and 24, illustrates the condition of the wound.

The course of the breast abscess was slow. On November 24, the skin on both sides had begun to close in but no appreciable change for the better was observable in the denuded breast tissue. A mass half the size of an orange protruded into the wound, both on the inner and outer sides. The mammary gland was still dissected out as previously, the granulation tissue on the surface being transformed, in islands, into a stony yellow material with pale, sluggish granulations between. The calcereous matter at times became loosened and could be picked off in pieces about 2 mm. thick. The hardening of the wound was not so marked as a week before. Along the outer margin of the gland the indurated areas remained as they were two week previously, rounded masses of uniform firmness. In the axilla the glands were somewhat swollen, one being, perhaps, as much as 4 cm. in diameter, but they were soft and did not feel as if calcified. The patient was much better; up and about the ward.

On December 5, the iodoform dressing was removed and the wound was packed loosely with sterile protective rubber strips.

On December 8, three days later, the nodules in the breast appeared to have diminished somewhat in size, while the calcium deposit in the wound seemed less marked.

Acting on the basis of the experiences of Gerhardt and Schlesinger who found that under a carbohydrate free diet, the calcium excretion was increased, the patient was put on the fixed carbohydrate free diet which is described later; this was continued for two weeks.

During this period, the healing of the wound progressed rapidly. On December 18, a note (W. S. T.) states: "Healing continues. The opening of the wound is now not more than 5 cm. in its greatest diameter. The calcified rim is smaller. The masses in the upper and outer quadrant of the breast have diminished in size and hardness. A few nodules are to be felt in the inner part of the breast, but they are very small; it is doubtful whether they have increased in number. Two axillary glands are also somewhat enlarged, the largest, just under the border of the pectoralis, is firm but does not feel stony." Five days later it was again noted (Dr. Thayer) that the calcified rim had softened considerably although there was still a good deal of stony material to be felt. The nodules in the outer part of the breast had diminished in size and firmness. From January 5 to 14, 1906, the patient was again put upon a carbohydrate free diet. By January 9 it was observed that the old calcified rim about the crater-like opening of the wound had wholly lost its stony hardness, having now the consistency of fibrous tissue. One large gland was to be felt in the axilla, while in the breast, over the fourth interspace, there was still a slight suggestion of a firm nodule. Two sinuses running back from the old incisions remained; in the anterior of these, gritty substance could be felt with the probe.

On January 15, there was a slight breaking down at a spot in the scar tissue

below the lower sinus. The wound was packed with iodoform gauze. On the following days there was a distinct deposit of calcareous material to be felt with the probe in the region where it had been in contact with iodoform gauze. On probing the wound the day before, no grating was to be made out. May this, possibly, have been a fresh deposition of calcium in new granulation tissue? Three days later, seventy-one days after the calcification was first observed, the old wound had entirely healed, the hard, sclerotic border having disappeared. The indurated masses in the breast were no longer to be felt. The glands in the axilla had diminished in size. On removing the dressing of the new opening there was a definite induration about the wound, especially in the lower part. No deposits of calcium could be seen or distinctly felt.

On January 21, a small piece of calcium ulcerated through near the lowest sinus. From this time on the sinuses continued to heal steadily. On February 8, but a few small glands were to be felt in the axilla, the largest not so large as the end of the little finger and of no especial firmness. There was no induration to be felt about the mouths of the openings of the sinuses. The patient was discharged, to return to the dispensary for the dressing of the sinuses. On one occasion, during the latter part of the patient's stay in the hospital, after packing a sinus, in which no calcium could be felt, with bismuth gauze, incrustations were found on the following day. It is not impossible, however, that this, as well as the appearance of calcium on January 16, was due to the ulcerating through of deeply seated deposits.

On May 19, the patient returned in response to a letter in order to be exhibited before the Society of Pathologists and Bacteriologists during the reading of this communication. She was perfectly well. The breast showed a puckered scar. There was no induration. The glands in the axilla had almost disappeared.

The results of the estimations by one of us (H. H. H.) of the intake and output of calcium during the several periods above referred to, are of considerable interest. At the outset, on December 3, the patient was put upon a simple diet of eggs and milk, the lime content of which could be easily estimated. It was intended that this diet should be persisted in for a period of a week or ten days but, owing to the strenuous objections of the patient, it was kept up only from December 3 to 8. Estimates of the calcium ingested in the food and excreted in the urine and fæces for three days, December 6 to 8 inclusive, showed a retention of 1.346 gms. or a daily average retention of 0.448.

From December 9 to 23, the patient was given the carbohydrate free diet which is described later. During twelve days, December 11 to 23, inclusive, a study of the intake and output of calcium revealed an excess of calcium elimination over the intake amounting to 2.6229, or a daily excess of calcium elimination amounting to 0.201.

From December 24 to January 6 a regular ward diet was allowed. Owing to the loss of a specimen the record of the calcium metabolism during this period was unfortunately lost.

From January 5 to 14, the patient was again upon a carbohydrate free diet, and during the eight days between February 7 and 14, the excess of calcium elimination over the intake was 1.050 gms., or a daily excess of elimination over intake of 0.131.

From January 27 to February 5, under ordinary ward diet, the intake and output of calcium were nearly equal; there was a retention of calcium amounting to 0.602 or a daily average calcium retention of 0.06 gms.

During the first period of carbohydrate free diet, crystals of calcium oxalate and phosphate were repeatedly found in the urine. During the second period, no calcium crystals were to be found. Diacetic acid and acetone were demonstrated in the urine on January 12 and January 13, but there was no especial increase in Ca elimination on those days.

It is undeniable that, while a diminution in the thickness of the calcified rim of the wound was noted on the day on which the carbohydrate free diet was begun, yet the greater part of the decalcification occurred during the time while the patient was on a carbohydrate free diet. During the two weeks from December 24 to January 6, while the patient was upon a regular diet, but little change was to be made out in the extent of the calcium deposits in the wound.

The association of the excess in Ca elimination with the absorption of the calcareous deposits in the wound and breast is an interesting and striking coincidence. While one may not be justified in assuming that the eliminated calcium represented that which was noted in the breast, it is but natural to suspect that the regime under which the excess in calcium elimination occurred had an influence upon the absorption of the deposits. The elimination of Ca in the urine was, it is true, no greater--indeed not so great—as in Gerhardt and Schlesinger's control case. It must, however, be remembered, in connection with this case, that we were not dealing with a healthy, normal individual, but with one who was convalescing from a long, wasting disease—a condition in which one

might not unreasonably expect to find an appreciable Ca retention. It may, therefore, be true that the excess of Ca eliminated during the periods of carbohydrate free diet, represents a change from the ordinary considerably greater than the figures themselves would suggest. The fact that during each of the intermediate periods there was a Ca retention of a remarkable degree during the first period when the diet was, it is true, of very high lime content, is striking in this connection.

It would be interesting to make further observations as to the intake and output of Ca during, and in the convalescence from, typhoid fever.

The conditions, general and local, under which the deposition of calcium is common are sufficiently familiar. The general conditions: in individuals in whom some process is going on involving the destruction of bone, such as caries, rapidly growing neoplasms and senility, the so-called calcium metastases.

The local conditions: such as caseating areas of tuberculosis in lungs, glands, etc., gummata, areas of regressive change in neoplasms, infarcts, atheromatous areas, old thrombi, the retained fœtus, the incrustation of cartilages and muscles around areas of venous stasis and chronic inflammation, the cardiac valves, pleural and pericardial exudates, hyaline degeneration in various regions, notably in the muscular coats of arteries, in ganglion cells, in the periphery of areas of cerebral softening, in disseminated fat necrosis, equal all conditions in which the affected areas are necrotic, degenerated, or of notably diminished vitality.

Especially interesting are the observations made by Litten who showed that a deposition of lime in the cortex of the kidneys followed temporary ligature of a renal artery in rabbits; a well-marked calcium deposit may be found after an occlusion of two hours' duration. Exactly similar changes have been described by various observers as the result of the ingestion of certain poisons, such as bichloride of mercury (Salkowski), subnitrate of bismuth (Langhans), acetate of lead (Binet), aloin (Gottschalk). Not all conditions, however, favoring necrosis, nor all poisons producing degenerative changes are followed by the deposition of lime salts.

Von Kóssa has recently made a study of the calcification-pro-

ducing poisons. Experimenting with a great variety of substances, he comes to the conclusion that those poisons which especially induce calcification in the rabbit are:

1. The salts of heavy metals (lead, bismuth, mercury and copper).
2. Of the metalloids, iodine and, perhaps, arsenic.
3. Among organic compounds, aloin and iodoform.

In susceptible animals poisoned by these substances the calcium is deposited in the shape of the phosphate; Von Kóssa has never found a deposit of carbonate. Not all the calcium is, however, present in the form of the phosphate; a part is apparently bound in some organic compound.

Inasmuch as the calcium content of the blood and bones of the poisoned (aloin) animal remains unchanged, while that of the urine is diminished, it is evident that the kidney fails to excrete the total amount of the calcium circulating in normal quantity, *i. e.*, the deposition is due to a calcium retention. The rabbit is particularly subject to the action of these poisons, owing probably mainly to the fact that its blood has normally an especially high calcium content. Von Kóssa has, further, demonstrated by the following interesting experiments that an increase in the quantity of the calcium in the circulation favors its deposition under the influence of these poisons. To one animal a certain amount of iodoform was administered by the mouth. To another, a smaller amount was given and, in addition, chloride of calcium subcutaneously. In the latter rabbit alone did calcification occur. The experiment was then repeated with three rabbits, one of which received iodoform alone, another an injection of chloride of calcium alone, and a third iodoform by the mouth and chloride of calcium subcutaneously. In neither of the first two rabbits was there any deposition of calcium. In the third there were marked changes. *In animals in which the calcium content of the blood has been artificially increased it is, therefore, possible to bring about calcification with doses of the poison essentially smaller than those required under ordinary circumstances.*

If one consider the course of events in the case which has just been described, a remarkable analogy appears between the conditions existing here and the experiments of von Kóssa.

1. In a patient suffering from hæmorrhage from the bowel in typhoid fever there were administered, in the course of eleven days, 132 grammes of lactate of calcium by mouth and five grammes of chloride of calcium subcutaneously.

2. In the area in which the subcutaneous injection of chloride of calcium was given there developed an extensive abscess from which Bacillus typhosus and Staphylococcus aureus were obtained.

3. After opening and draining the abscess was packed with iodoform gauze.

4. Extensive areas of calcification developed in the granulation tissue wherever it was exposed to the action of iodoform as well as in other adjacent parts of the breast.

5. It is possible that a similar deposition of calcium followed the dressing of subsequently developed sinuses with subnitrate of bismuth.[3]

In other words, in an individual in whom the calcium content of the organism was probably increased as a result of the administration by mouth and hypodermically of lactate and chloride of calcium, an abscess developed which was dressed with iodoform gauze; this was followed by the deposition of calcium in the granulation tissue immediately adjacent to a substance (iodoform) which is known, experimentally, to favor the deposition of calcium in rabbits. This deposit of calcium disappeared rapidly and completely under a carbohydrate free diet. During three weeks of this period the average daily excess of calcium elimination over that of calcium intake amounted to 0.174 + gr.

We have been unable to find a similar case in literature. Interesting and suggestive in this connection are the observations of Schujeninow who, with Chiari, demonstrated the deposition of calcium in muscle fibres about the borders of laparotomy wounds in seventeen out of twenty-four cases. In the earliest case examined, calcification was discovered thirteen hours after the incision. This reached its height from about the ninth to the twentieth day and then disappeared by absorption. It was noted that the calcification

[3] Although the sequence of events here was remarkable, we are rather inclined to regard the appearance of the calcium after the beginning of the bismuth dressings as due to the breaking through of deeper lying calcium masses.

was always near the ligature and occurred in fibres which had undergone colloid degeneration following the disturbances of circulation as a result of the ligatures. It would be interesting to know whether, in these cases, the wounds were dressed with iodoform. In his experiments with rabbits the wounds were closed with iodoform collodion.

As to the cause of this extensive deposition of calcium our observations do not justify a definite conclusion. One can only point to the remarkable combination of circumstances—the extensive calcification of the granulation tissue and of circumscribed areas throughout the breast after an acute abscess developing at the seat of an injection of calcium chloride in an individual who had been taking, for eleven days, large doses of the lactate of calcium—and further to the fact that the calcification did not appear at the outset, but only after the wound had been packed with gauze impregnated with iodoform, a substance which, experimentally, has been found in some animals to favor the deposition of calcium.

We have endeavored, in several instances, to reproduce this picture in rabbits by administering lactate of calcium subcutaneously, after which an abscess was to be produced, opened and packed with iodoform gauze.

Five rabbits in all were used. Daily injections of 0.13 per 500 grammes weight were administered for ten days. In attempting to produce an abscess, the following method was evolved. Aleuronat meal in from seven to ten times its weight of water to which 3 per cent. starch was added, was injected under the skin. Failing to produce an abscess in this manner, dead cultures of Bacillus typhosus were used with fatal results. One abscess occurred spontaneously and one was obtained by the hypodermic injection of croton oil in ether. Although both were packed with iodoform gauze, in neither case was there a deposit of calcium. No trace of calcium was found on necropsy in kidneys or liver.

Determinations of the intake and output of calcium.—These analyses were made by one of us (Dr. Hazen)[4] in the clinical laboratory of the John Hopkins Hospital under the direction of Dr. C. P. Emerson, according to the following method:

[4] I desire here to express my indebtedness to Dr. Emerson and Dr. Boggs for their assistance in this work. H. H. H.

At the outset the patient was given a teaspoonful of charcoal by mouth. Beginning with the meal following this, a quantity of food exactly similar to that given by mouth was set aside for analysis. The first stool after the charcoal had appeared in the fæces was saved; the urine was collected as soon as the charcoal had appeared. To close the period, charcoal was again administered, after which no food was kept. After the appearance of this charcoal in the fæces, neither urine nor stools were saved.

Great care was taken to insure that equal amounts of the same food were saved for examination; this was personally watched. The food was accurately weighed and dried in a thermostat. The total food for the period was then repeatedly run through a sausage grinder, and when thoroughly mixed, samples were removed for the determination of the Ca. This was done by burning the food until a perfect ash was secured; it was then dissolved in HCl, and the same procedure was followed as in the case of the urine. As it was naturally impossible to get an exact half-portion of the food in the case of the eggs, the following method was adopted. One dozen eggs were analyzed and the mean Ca content used as a basis for estimation. It was found necessary to boil the eggs hard before burning them.

All food analyses were made in triplicate, and in no instance was there the slightest variation in the Ca determination, good evidence that a uniform mixture was secured.

In the case of the stools the same method was employed as for the food, except that they were analyzed in daily quantities. Analyses were made in duplicate.

The urine was saved in twenty-four hour amounts, preserved with chloroform and estimated separately. At first the estimation was made according to the well-known gravimetric method described in the tenth edition of Neubauer and Vogel, p. 746. It was, however, soon found that, in about 15 per cent. of the cases, the Ca was not precipitated, necessitating the use of another specimen. The following modification was accordingly devised which by careful duplicate, comparative tests with the older method, proved entirely reliable, while giving invariably a precipitate of oxalate of calcium.

Two hundred cubic centimetres of the urine were employed. The urine was then acidified with HCl and filtered; NH_4OH was added until a precipitate formed. This suspension was neutralized with acetic acid instead of HCl. An excess of ammonium oxalate and a few c.c. of sodium acetate were then added, and the amount put in an evaporating dish. This was covered with a glass plate and heated over night on a water bath. It was then filtered through an ashless filter paper, until the filtrate was perfectly clear. The precipitate was washed with hot water and burned with the paper in a blast flame, in a platinum crucible until it lost no more weight. This converted the oxalate into the oxide, in which form it was gravimetrically determined. Tests were made in duplicate.

During an initial period in order to determine what she was doing upon a more or less normal mixture the patient was put upon a milk and egg diet. The diet consisted of 375 c.c. of milk and two eggs every four hours. As the patient objected strenuously to its

continuance it was abandoned after three days. In the light of further work it would seem that this diet was unfortunately chosen, as the Ca content of milk is so high that, in the large quantities administered, it was probably partly responsible for the appreciable Ca retention which was demonstrated. The results of the estimates of the intake and output of calcium during this period are shown in the record of Period I in the table. As has been already noted, there was a considerable Ca retention.

Table Showing the Results of Estimates of the Intake and Output of Calcium.

	Ca in Urine.	Ca in Stools.	Total Ca Excretion.	Ca in Food.	Ca Retention.	Ca Excess Elimination.	Daily Average Amount Ca in Urine.	Per Cent. of Ca Excreted in Urine.	Relation of Ca in Urine to that in Stools.	Daily Average Excretion of Ca.	Daily Average Amount of Ca in Food.	Daily Average Retention of Ca.	Daily Average Excretion of Ca in Excess.	Per Cent. of Ca Excreted in Stools.
Period I. 6/xii–8/xii, Inclusive. Milk and Eggs Diet.	.615	5.340	5.955	7.301	1.346	—	0.205	10.32%	11.5:100	1.985	2.433	.448	—	89.67%
Period II. 11/xii–23/xii, Carbohydrate Free Diet.	3.094	4.561	7.655	5.033	—	2.622	0.238	40.4 %	65.6:100	0.588	0.387	—	0.201	59.58%
Period III. 7/i–14/i. Carbohydrate Free Diet.	1.572	4.485	6.057	5.007	—	1.050	0.196	25.95%	35:100	0.751	0.6258	—	0.131	74.04%
Period IV. 27/i–5/ii, Normal Mixed Diet.	1.260	9.849	11.109	11.711	0.602	—	.126	11.34%	12.7:100	1.1109	1.1711	0.06	—	88.65%

From December 11 to 23 the patient was put upon a carbohydrate free diet, essentially the test diet advised by v. Noorden in diabetes.[8] An exact half-portion of every meal was measured and set aside for analysis. The record of Period II on the table shows a reversal of the picture in Period I—a high per cent. of Ca elimination in the urine and a distinct excess in elimination of Ca over the intake. During this period crystals of oxalate and phosphate of lime were repeatedly found in the urine. Acetone and diacetic acid were not demonstrable.

From December 24 to January 6 inclusive, the patient was on ward diet, but, unfortunately, the Ca was not determined, owing to loss of a specimen.

[8] Breakfast: 5 grm. (⅙ oz.) tea steeped in 200 c.c. (6⅔ oz.) water; 150 grm. (5 oz.) ham; one egg.

Lunch: 200 grm. (6⅔ oz.) cold beef; 60 grm. (2 oz.) fresh cucumber (or good quantity of lettuce) with 5 c.c. (teaspoonful) vinegar; 10 c.c. (2

From January 7 to 14 inclusive, the patient was again upon a carbohydrate free regime. During this period no crystals of oxalate of calcium were found in the urinary sediment.

On January 12 and 13, acetone and diacetic acid were found in the urine. There was, however, no increase in Ca elimination in the urine on those days. The record of Period III in the table shows again an excess in the Ca eliminated, though not so marked as in the previous period.

From January 27 to February 5, a study of the calcium intake and output was made, while the patient was on ordinary ward diet. Here, again (Table, Period IV), a slight Ca retention is demonstrable with a marked diminution in the per cent. of Ca eliminated by the urine.

A study of the table shows that the excess of calcium was excreted by the urine, the per cent. of calcium excretion in the stools falling strikingly during the period while the patient was upon a carbohydrate free diet.

It is unfortunate that the relations between the ammonia and calcium output should not have been determined. In the observations of Gerhardt and Schlesinger the ammonia output was found to run parallel with that of the calcium.

The patient remained in excellent health throughout the experiments. She gained materially in weight during the whole period. The weight was taken regularly during the experiment, but owing to an unfortunate misunderstanding, the entries were not regularly made upon her temperature chart. There was a slight loss of not more than a pound or two during each of the periods of carbohydrate free diet, with considerable gains during the intermissions. Thus, on November 26, the weight was 82½ pounds. On December 3, the day that the milk and egg diet was begun, the weight was 86½ pounds. Two weeks later, December 17, in the middle of the first carbohydrate free period, the weight was 84 pounds. A month later, on January 14, at the end of the second carbohydrate

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teaspoonfuls) olive oil and salt and pepper to taste; 20 c.c. (4 teaspoonfuls) brandy with 400 c.c. (13–14 oz.) Apollinaris water (soda water or ordinary water will do); 60 c.c. (2 oz.) of coffee without milk or sugar.

Dinner: 200 c.c. (6⅔ oz.) clear bouillon; 250 grm. (8⅓ oz.) beef (weighed raw) basted with 10 grm. (⅓ oz.) butter; 8 grm. (2⅔ oz.) green salad with 10 c.c. (2 teaspoonfuls) vinegar and 20 c.c. (4 teaspoonfuls) olive oil or 3 tablespoonfuls of some good, well cooked vegetable such as spinage; 3 sardines; 20 c.c. (4 teaspoonfuls) brandy with 400 c.c. (13–14 oz.) Apollinaris water (ordinary water or soda water will do).

Supper: 2 eggs, raw or cooked; 400 c.c. (13–14 oz.) seltzer water (or any good water).

free period, the weight was 103 pounds. On February 8, the day of discharge, the weight was 114 pounds.

* * *

SUMMARY.

1. In a patient with typhoid fever, who had taken, during eleven days, 132 grammes of lactate of calcium by the mouth, as well as 5 grammes of chloride of calcium subcutaneously, there developed, eleven days after the subcutaneous infusion, a large abscess of the breast from which Bacillus typhosus and Staphylococcus aureus were obtained on culture. The abscess was opened and the wound packed with iodoform gauze. Eleven days later a deposit of calcium appeared in the granulation tissue bordering the wound. This rapidly increased until the rim of the opening and the lining granulation tissue were converted into a hard, calcareous mass. A number of nodules of an apparently similar character appeared in other parts of the breast.

2. A month later the iodoform packing was removed and the patient was put on a carbohydrate free diet. This was continued for twenty-four of the next thirty-six days. Under this treatment the abscess almost entirely healed with complete disappearance of the deposit of calcium.

3. Studies of the intake and output of calcium showed that, during three days under a diet of milk and eggs there was a material calcium retention (1.346 grammes): (*b*) That during two periods amounting to three weeks in all, under a carbohydrate free diet, there was an excess of Ca elimination over the intake of 3.672 grammes. (*c*) That during ten days following the last carbohydrate free period, under a normal diet, there was a retention of calcium amounting to 0.602 gramme.

4. The disappearance of calcium from the breast was associated in time with the carbohydrate free diet and the excessive elimination of calcium.

5. Under the carbohydrate free diet acetone and diacetic acid appeared on two occasions in the urine, but were not accompanied by any increase in the elimination of calcium.

6. With regard to the cause of the calcification, we cannot speak with certainty. The remarkable association of the deposition of

calcium at the seat of an injection of calcium chloride in an individual who had been receiving large quantities of lactate of calcium by the mouth—a deposition occurring in tissues which were in direct connection with or in the immediate neighborhood of a dressing containing iodoform, a poison which in certain animals produces degenerative changes in the kidneys and liver which are peculiarly prone to calcification, is striking and worthy of note.

7. In how far the decalcification of the affected area may have been due to the carbohydrate free regime with the consequent excess in calcium elimination, it is impossible, on the basis of a single case, to say. The coincidence in time of the disappearance of calcium from the breast with this treatment is, however, remarkable and extremely suggestive.

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TOXINS AND ANTITOXINS—SNAKE VENOMS AND ANTIVENINS.

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In this article we shall give an account of some quantitative determinations of the relation between the different snake venoms and their specific antivenins. The venoms employed were those of the cobra (*Naja tripudians*), of *Crotalus adamanteus* and of *Ancistrodon piscivorus*. Dr. George Lamb of India has kindly furnished us with cobra venom. The other two were personally procured by one of us (N.). The cobra antivenin was obtained through the kindness of Professor Calmette of Lille. The other two antivenins were prepared at this Institute.

McFarland[1] was the first to attempt the preparation of crotalus antivenin. In this, it appears, he had considerable difficulty, because the animal he used, the horse, reacted to subcutaneous injection with widespread œdema and inflammation. By the use of intravenous injection of unaltered venom McFarland obtained an antivenin which, according to his account, was protective against neuro-toxin, but had scarcely any effect on the irritative principle.

In order to avoid the inherent difficulty of immunization with unmodified venom, Flexner and Noguchi[2] attempted to eliminate the local effects by the use of a dilute solution of hydrochloric acid and of trichloride of iodine. They obtained in this way, in experiments on dogs and rabbits, an antivenin which neutralized perfectly the toxic effects of all the elements of crotalus venom.

In this Institute a *goat* was used, which was injected with unmodified crotalus venom subcutaneously. The animal stood the injection well; œdema was slight, and had disappeared at the end of a few days after each injection. The general condition of the

[1] McFarland, *Proc. of the Soc. of Amer. Bact.*, Dec., 1900.
[2] Flexner and Noguchi, *Jour. Med. Research*, 1903, vi, 363.

animal was apparently unchanged, and its weight slowly increased. (See Table I.)

It will be seen from reference to the protocol of the experiment that the highest antitoxic power was found on March 23 (see Table II) when one cubic centimeter of the serum neutralized the effects of about six minimal lethal doses. A little later the amount of the antitoxin diminished in spite of injections of relatively large doses of the venom. The antitoxin used in the following experiments was obtained on March 30 and 31, 1904.

On May 11, 1904, two kids were born and the amount of antitoxin in the colostrum was determined. The quantity of antitoxin was about the same both in the blood and in the milk. This fact is of considerable interest inasmuch as previous experiments[3] had shown that the amount of antitoxin in the milk was much less than that in the blood. The milk of non-immunized goats was also examined as controls and was found to be devoid of antivenomous action.

Experiments on immunization against the venom of the *water moccasin* were first undertaken with *unmodified* venom injected *subcutaneously*. The goats appeared to stand the injections of small amounts very well, but in two cases (Tables III and IV) the animals died after the injection of 0.2 gram. They had previously tolerated repeated injection of smaller quantities.

To determine whether 0.2 gram was the m. l. d. for goats of this size, 0.15 gram of moccasin venom was injected subcutaneously into a normal goat. But, as shown in the protocol of the experiment (Table V), this animal died after a single subcutaneous injection of 0.15 gram. Then we used the moccasin venom *modified* by placing 0.5 gram of the dried venom, dissolved in one hundred cubic centimeters of one per cent. hydrochloric acid, in the thermostat at 37° C. for twenty-four hours. The solution was neutralized with sodium hydroxide. Immunization with this modified venom was rapidly and easily accomplished (Table VI). A trial serum, drawn on May 13, 1904, showed a weak antivenomous power, but after continued injections of the venom the antitoxic

[3] Salomonson and Madsen, *Ann. de l'Inst. Pasteur*, 1897, xi, 315, and 1899, xiii, 262.

standard rose to a point at which, on May 30, 1904, one cubic centimeter of the serum completely neutralized all effects of 0.0024 gram (2 m. l. d.) of the unmodified venom (Table VII). This antivenin was used in the experiments which are to be discussed later.

Solutions of the venoms were made as follows:

0.5 grm. dried crotalus venom in 100 c.c. water,
0.5 grm. dried moccasin venom in 100 c.c. water,
0.4 grm. dried cobra venom in 100 c.c. water.

These solutions preserved under toluol at a temperature of from 2° to 3° C. were used for all the experiments which follow. The two first solutions showed no perceptible deterioration during the month of experimentation, but the toxicity of the cobra venom was sensibly diminished.

CROTALUS VENOM.

The determination of toxicity was generally made by intraperitoneal injections into guinea-pigs. Table VIII, presenting a small series of experiments, shows in detail the relation between the weight of the animal and the toxicity of the venom. Apparently this relation remains about the same per kilo for animals of from 250 to 500 grams, while smaller animals weighing about 125 grams are much more sensitive. Crotalus venom *passed through a Chamberland filter always loses more than fifty per cent. of its toxicity,* as shown in Table IX. The toxicity is considerably lower and the action is much more irregular when the venom is injected *subcutaneously* (Table X). White rats are very resistant to its action (Table XI). Tables XII and XIII give the details of experiments dealing with the *neutralization of crotalus venom by its specific antivenin.*

To 0.006 gram of venom were added the amounts of antivenin shown in the first column of Table XIII. The mixtures were kept two hours at 37° C. and then injected in the fractional quantities indicated in the second column of the table. Taking the dose which kills after from fifteen to seventeen hours as a unit, we have derived the facts shown in Table XIV, where under "*n*" are indi-

cated the quantities of antivenin added to a constant dose of venom, 0.006 gram, and under "q observed," the observed toxicity.

These observed values, after allowing for errors in experiment, can be expressed by the same formula, which represents the combinations of toxins and antitoxins of other substances.[4]

$$\frac{\text{Free toxin}}{\text{vol.}} \cdot \frac{\text{Free antitoxin}}{\text{vol.}} = K \cdot \text{Toxin-antitoxin.}$$

In this case:

$$\frac{1}{q_0}\left[n\frac{1}{q}\cdot p - \left(\frac{1}{q} - \frac{1}{q_0}\right)\right] = K\left(\frac{1}{q} - \frac{1}{q_0}\right)^2$$

where $1/q$ represents the quantity of antitoxin equivalent to an amount of toxin used and K the constant of association.

With $p = 1$ and $K = 0.0048$, we found the value of q, shown under "q calc. I." For greater convenience these results are graphically represented in Fig. 1. The tracing shows the calculated values of "q," while the dotted circle shows the observed values. It will be noted, therefore, that the values derived from the experiments mentioned above can be expressed according to the simple formula:

$$-\frac{\delta q}{dn} = Kq.$$

We have shown that this formula represents the action of saponin and cholesterin[5]; the column under "q calc. II" shows the correspondence.

The experiments on *rabbits* (Table XV) showed a marked difference between subcutaneous and intravenous injections of venom. For the *neutralization experiments* shown in Table XVI, intravenous injections only were used. The technique was the same as that used in the experiments with guinea-pigs, the quantity of venom being always 0.006 grm. The results obtained by taking as a unit of toxicity a dose killing after five days are summarized in Table XVII. They are shown in Fig. 1 by a dotted square. The form of the tracing obtained agrees to a certain extent with that

[4] Festkrift ved Indvielsen af Statens Serum Institut, Copenhagen, 1902.
[5] Extr. du Bul. de l'Acad. Royale de Danemark, 1904.

determined for guinea-pigs; further calculations were omitted, since the first part of the determination showed an error.[6]

Antivenin produced by immunization has also the power of

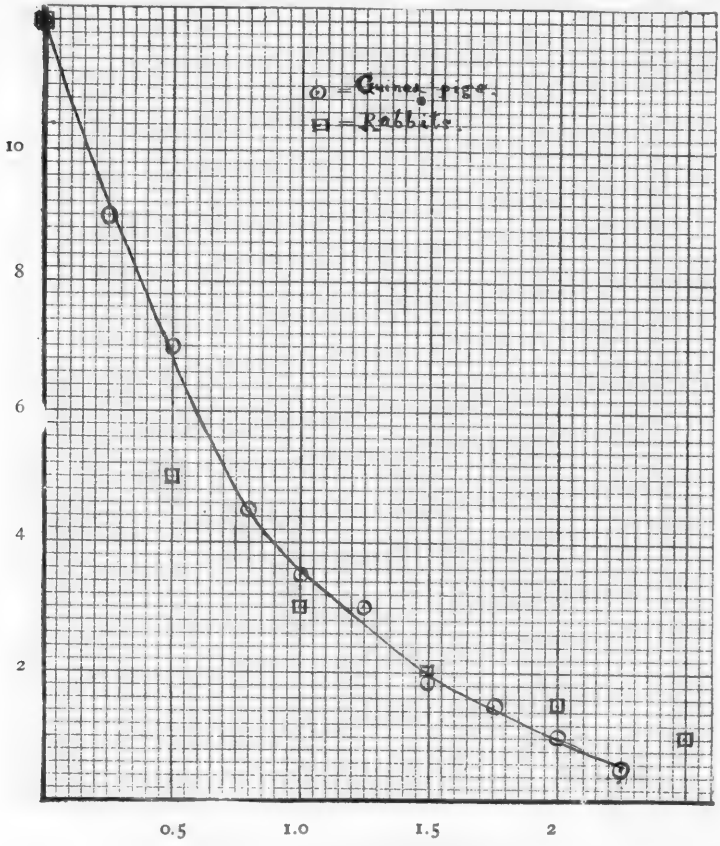

FIG. 1. Crotalus venom-antivenin.

neutralizing the hæmolytic property of crotalus venom. Table XVIII gives the details of experiments; one cubic centimeter of

[6] Perhaps these errors are due to the different methods of injection. At the time when these experiments were carried out, the researches of Morgenroth (*Berliner klin. Woch.*, 1904, xli, 526) on the slow combination of toxin and antitoxin of diphtheria had not been published. Nevertheless, it does not seem unlikely that the reaction will be finished after two hours at 37° C.

0.05 per cent. crotalus venom mixed with the quantities of crotalus antivenin indicated under "*n*" was added to 0.9 per cent. sodium chloride solution to bring the total volume to two cubic centimeters. These mixtures were kept two hours at 37° C. and then their

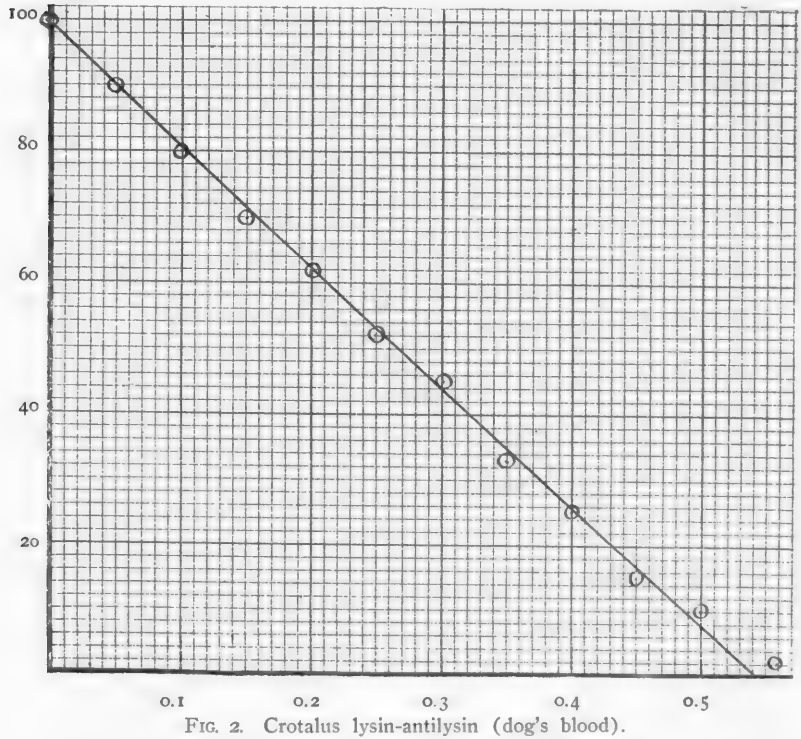

FIG. 2. Crotalus lysin-antilysin (dog's blood).

hæmolytic action, on a five per cent. suspension of dog's blood in 0.9 per cent. saline solution, was examined by the method used in this Institute. In Table XVIII, columns marked "I, II, III," show the doses producing equal hæmolysis; the averages, representing the hæmolytic power, have been estimated from these figures and are given under "*q* obs." As shown graphically in Fig. 2, the form of tracing obtained by neutralization is, in this case, practically a straight line.[7]

[7] The constant of equilibrium was, in this case, almost zero. In the table are shown, under "q calc.," the values estimated on the assumption that 1 c.c. of

COBRA VENOM.

The first determinations of the m. l. d. were made by subcutaneous injections of 0.2 per cent. solution of venom into *guinea-pigs* weighing from 650 to 670 grams. (Table XIX.) A little later another solution of venom, 0.4 per cent., filtered through a Chamberland filter (Table XX) was examined. A comparison was, however, somewhat difficult, because the animals of the two series were not

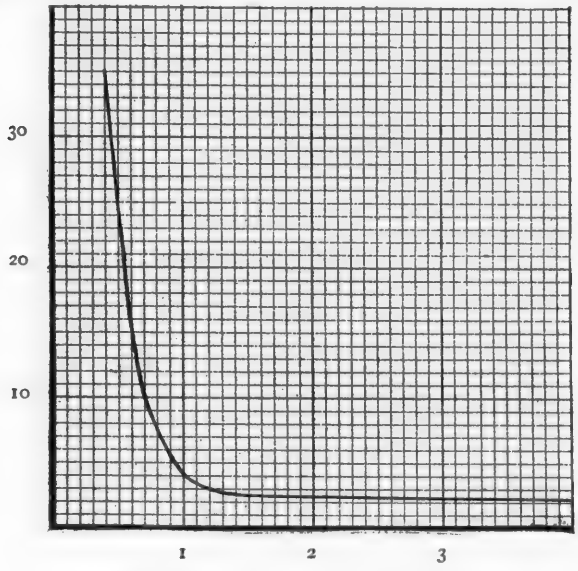

FIG. 3. Toxicity of cobra venom.

of equal weight; it was seen, nevertheless, that filtration did not perceptibly diminish the toxicity of the solution.

The relation between the dose and the toxicity is shown by the tables. In the first place, the time of death is greatly shortened up to a certain point as the dosage is increased. With 0.0005 gram it is 3.75 hours, but beyond this point an increase of the dose does

this antivenin is equivalent to 1.86 c.c. of the solution of crotalus venom. Moreover, for 0.55 c.c. of antivenin there was a discrepancy, pointing to a feeble dissociation of the venom-antivenin combination, corresponding practically to the value of "K" (the constant of dissociation) of 0.0006.

not proportionally shorten the time intervening between the injection and the death of the animal.

In Table XXI are recorded, for the greater part, the same experiments shown in Tables XIX and XX, undertaken, however, with a little larger animals (marked *). If it be arbitrarily assumed, as in a previous paper,[8] that 0.0005 gram is equal to one m. l. d., the results will group themselves with a certain regularity about this dose. Perhaps this will appear more clear by reference to Fig. 3, where the m. l. d. is drawn on the axis of the abscissa, while the time, in hours, is constructed on the ordinates. It will be seen that the determinations form a very regular curve of an asymptotical type. The decrease in time does not agree with the increase in dose; this decrease is perhaps due to the time of incubation and to the method of injection, since it is well known that venom requires some time for absorption from subcutaneous tissues. With intravenous injection the decrease would probably have been much more pronounced. It is evident that the values obtained with doses ranging from 0.6 to 1.2 m. l. d. give the most decisive determinations. The lower and very much larger doses show results which are much less certain. The method just described has, moreover, the advantage that it is not always necessary to find out the dose which will kill within a fixed time. After determining the scale once one can find by interpolation which fraction of the minimal lethal dose is present in a certain mixture.

On account of the very important rôle which lecithin plays in the hæmolysis caused by cobra venom, some experiments were undertaken in order to learn whether one cubic centimeter of a 1/50 *N* solution of lecithin would produce any appreciable change in the toxicity for guinea-pigs. As shown by Table XXII, no such change occurs.

The combination of cobra venom and its specific antivenin seems to occur at 36° C. very rapidly. The small series of experiments, shown in Table XXIII, does not indicate any appreciable difference in the neutralizing power, whether venom and antivenin are mixed together and injected immediately or injected at the end of six

[8] Arrhenius and Madsen, *Acad. Roy. des Sciences et des Let. de Danemark*, 1904.

hours.[9] The mixtures of toxin and antitoxin were incubated for two hours at 36° C.

During the experiments undertaken to explain the neutralization of venom with antivenin, it appeared that the first preparations of antivenin which were at our disposal were too feeble. In Table XXIV will be found in the second column, under "*n*," the quantities of serum (Antivenin I), in cubic centimeters, which were added

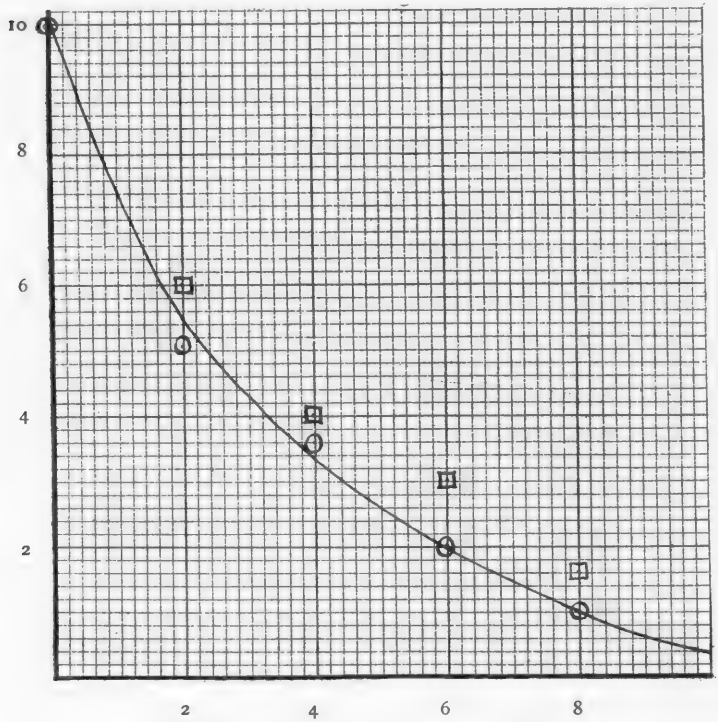

FIG. 4. Cobra venom-antivenin (guinea pigs).

to 0.008 gram (16 m. l. d.), and to 0.004 gram respectively of the venom. In the following column is shown the fraction of this

[9] The experiments were not complete. Experiments with intravenous injection were also lacking, and would be necessary to make it perfectly clear that the reaction between venom and antivenin is rapid (cf. the researches of Morgenroth on diphtheria antitoxin, *loc. cit.*)

mixture which was injected; and in the last two columns the results. One experiment with a new antivenin (II) showed that from ten to twelve cubic centimeters would neutralize the effect of 0.004 gram. It was necessary to know that this antivenomous effect was not due in part to the normal serum of the horse. In one ex-

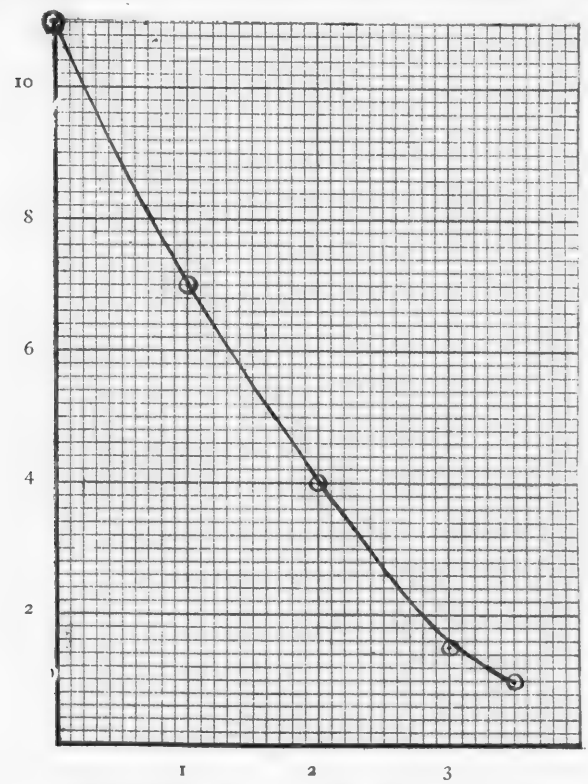

FIG. 5. Cobra venom-antivenin (guinea pigs).

periment twenty cubic centimeters of normal horse serum gave almost no antagonistic effect when tested against 0.008 gram of the venom (Table XXV). With this antivenin (II) (Table XXVI) some experiments upon partial saturation were undertaken. To 0.0028 gram of the venom were added the amounts of antivenin indicated under "*n*." The mixtures were, as usual, incubated

for two hours at 36° C.; then the fractions indicated in the following column were injected intraperitoneally into guinea-pigs. There were series of experiments, one with animals of 370 grams, another with those of 450 grams. The determinations of toxicity, q, in the last series (B) will be found in Table XX. They can be summarized as follows:

n	q
0	10
2	5
4	3.5
6	2
8	1.7
10	< 1

Fig. 4 furnishes a graphical representation of these results.

In one series (guinea-pigs weighing 370 grams) there was no determination made for $n = 0$. The other values are marked by a dotted square in Fig. 4. It will be seen that this curve corresponds very well to the first one.

The last series of *neutralization* experiments were carried out in June, 1904, with an antivenin considerably stronger than that previously used (Table XXVII); four cubic centimeters of this antivenin neutralized effectively 0.003 gram of venom. The results of these experiments may be summarized as follows:

n	q
0	11
1	7
2	4
3	1.5 (?)
4	< 1

The tracing in Fig. 5, which graphically represents the results of neutralization, is not very strongly curved. The results of neutralization of the *hæmolysin of cobra venom* by its antivenin was tested twice with Antitoxins I and II. The procedure was that which we have usually employed: one cubic centimeter of a 0.1 per cent. solution of cobra venom was mixed with various quantities of antivenin; to these mixtures was further added a sufficient amount of physiological salt solution to bring the total volume to two cubic centimeters. A mixture, kept for two hours at 37°, was

measured into tubes and eight cubic centimeters of a one per cent. suspension of horse's blood corpuscles were rapidly added (each liter of the suspension contained eight cubic centimeters of 1/100N lecithin solution). The tubes were incubated for two hours at 37° C. and then over night at a lower temperature; hæmolysis was estimated by our usual method.

The figures in Table XXVIII are the averages, derived in the

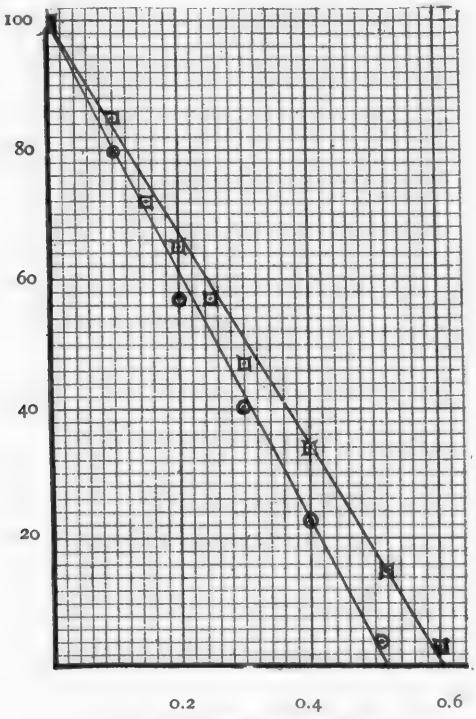

FIG. 6. Cobra lysin-antilysin.

same way as those of the experiments recorded in Table XVIII. In Fig. 6 the observations with Antivenin I are marked by a dotted circle, and with Antivenin II by a dotted square. The tracings, as may be seen, are rather close to straight lines.

The theoretical values of "q calc." are calculated under the sup-

position that one cubic centimeter of Antivenin I is equivalent to 1.98 c.c. of venom solution, and of Antivenin II equivalent to 1.7 c.c. of venom ($K=0$). For both series, the last values for "q obs." are then considerably lower than the calculated ones. This indicates that there is a clear dissociation[10] (corresponding to $K=0.0016$). Thus it is not quite correct to assume that the curve of neutralization of cobrolysin and of cobra antilysin is a completely straight line.[11]

WATER MOCCASIN VENOM.

The toxicity of water moccasin venom was examined on two occasions, May 6 and August 22, 1904; during the interval of one hundred and eight days the venom was preserved at from 2° to 4° C. The animals employed for these experiments were guinea-pigs weighing 260 grams. The venom was injected into the peritoneal cavity. A comparison between Tables XXIX and XXX shows that the toxicity was intact. The experiments upon the *neutralization of this venom by its specific antivenin* show singular results. They were carried out by mixing 0.012 gram of venom,—about 10 m. l. d.—with varying amounts of antivenin. After the mixture had been kept for three hours at 37° C. its toxicity was tested by injecting various fractions into guinea-pigs weighing 250 grams. (Table XXXI.) By the addition of 2 c.c. of serum the toxicity was lowered from 10 to 6; of 4 c.c., to about 4 or 5; of 5 c.c., to 3; and of 6 c.c., to about 2.5. But by the addition of still larger amounts of antivenin no further reduction of the toxicity could be produced, as experiments with 8, 9, 10, 20 and 40 c.c. of antivenin showed. This phenomenon was perhaps due to the fact that the antivenin in large enough doses had in itself a toxic action.[12] Unfortunately, our stock serum was exhausted, and we were unable to carry further an investigation of this phenomenon.

Finally, a study was made of the *hæmolytic* action of the venom of the water moccasin; one cubic centimeter of this venom (stock

[10] Myers, *Jour. Path. and Bact.*, 1900, vi, 415. Flexner and Noguchi, *Jour. Path. and Bact.*, 1903, viii, 379.

[11] Kyes, *Berl. klin. Woch.*, 1904, xli, 494.

[12] In fact, it is well known that the normal serum of the goat is often toxic for quinea-pigs.

solution) was mixed with different quantities of moccasin antiserum. The mixtures were incubated for two hours at 37° C. Special experiments had shown that the reaction was complete at the end of ten minutes. The hæmolytic tests were made in the usual way, using a five per cent. suspension of dog's blood. The results are given in Table XXXII and in Fig. 7. It will be seen

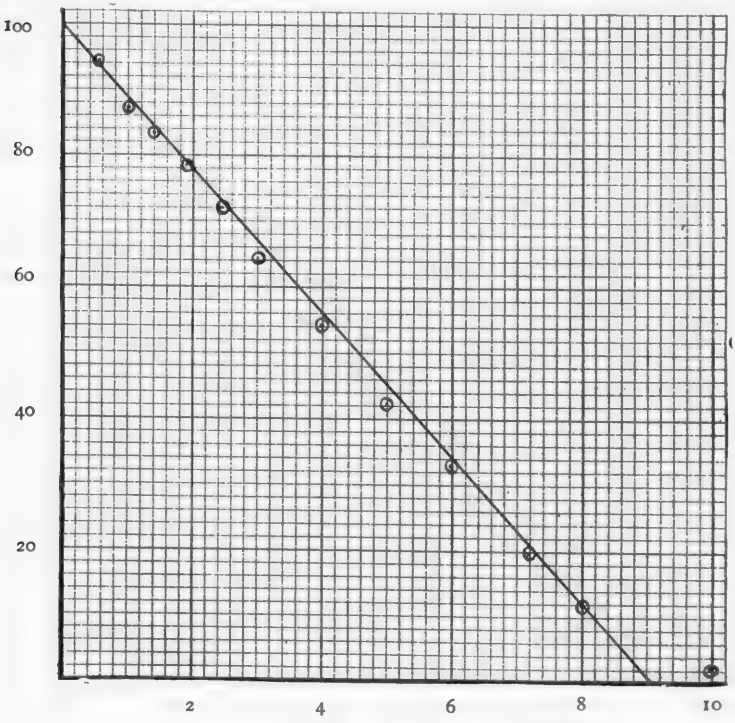

FIG. 7. *Ancistrodon piscivorus* lysin-antilysin.

that the tracing representing the combination is approximately a straight line, except for high concentrations of antitoxin. The values are estimated on the supposition that one cubic centimeter of antivenin is equivalent to 1.2 cubic centimeters of the venom solution, and that $K = 0$. In this case the values calculated for higher concentrations show more variation than in the case of crotalus and cobra lysins. K should be about 0.006.

Unfortunately, the lack of material prevented the continuation of our experiments. Those given are to be regarded as preliminary on account of their small number. For this reason we have not presented any general conclusions, but have given our experiments in the hope that they may prove the starting point of similar researches in the future.

SUMMARY.

A specific antivenin against crotalus venom can be prepared by the immunization of goats. A specific antivenin against water moccasin venom can be produced by the immunization of goats with this venom, modified by hydrochloric acid. Immunization with the unmodified venom is very difficult.

The toxicity of crotalus venom is diminished more than fifty per cent. by passage through a Chamberland filter. There is a simple relation between the toxicity and the body weight for guinea-pigs weighing from 250 to 500 grams. Smaller guinea-pigs (125 grams) are comparatively less resistant. The toxicity is smaller by subcutaneous than by intraperitoneal injection (guinea-pigs), or by intravenous injection (rabbits). White rats are very resistant.

The toxicity of cobra venom is not measurably diminished by filtration through a Chamberland filter. The relation between the amount of venom and the corresponding time of death is very regular, and can be expressed by a curve of asymptotic nature. Lecithin does not increase the toxicity.

The tracings representing toxin-antitoxin neutralization for the three venoms (crotalus, cobra and moccasin) show deviation from the straight line. This deviation is most pronounced for the toxic quota of the venoms. The tracing representing crotalus venom-antivenin neutralization, determined on guinea-pigs, can, within errors of experiment, be expressed by the equation:

$$\text{Free toxin} \cdot \text{Free antitoxin} = K \cdot \text{toxin-antitoxin}.$$

The corresponding tracing determined on rabbits is somewhat different, but both tracings are much more markedly curved than that for cobra venom-antivenin. The neutralization tracing of water moccasin venom shows the peculiarity, that small amounts of anti-

TABLE I

IMMUNIZATION OF A GOAT WITH CROTALUS VENOM.

Nov.	4, 1903,	0.0001 grm.	
	7	0.0002	
	10	0.0004	
	13	0.0008	
	16	0.0012	
	20	0.0016	
	23	0.002	
	26	0.0025	
	30	0.003	
Dec.	4	0.0035	
	9	0.004	
	12	0.004	
	16	0.005	
	21	0.008	
	26	0.01	
	31	0.015	
Jan.	2, 1904,	0.02	
	6	0.032	
	13	0.04	
	16	0.04	
	19	0.05	
	23	0.08	
	28	0.1	
Feb.	2	0.14	
	10	0.16	
	15	0.2	Slightly ill.
	23	0.05	Blood was drawn before the injection (1 c.c. of this serum protected against 2.5 m. l. d.).
	29	0.1	
Mar.	4	0.2	Slight swelling around the site of injection.
	9	0.25	
	15	0.3	
	19	0.35	
	23	0.4	Bled (1 c.c. protected against 6 m. l. d.).
	30		Bled about 350 c.c. (1 c.c. protected against 5 m. l. d.).
	31		Bled about 500 c.c. (1 c.c. protected against 5 m. l. d.).
Apr.	18	0.05	Bled (1 c.c. protected against 0.4 m. l. d.).
	20	0.1	
	24	0.15	
	27	0.2	
	30	0.35	
May	6		Bled (1 c.c. protected against 4 m. l. d.).
	7		Bled (1 c.c. protected against 3.2 m. l. d.).
	11		Two kids born. The milk was tested and it was found that 1 c.c. protected against 2.4 m. l. d.

venin decrease the toxicity to a minimum, but the toxicity is again increased by further addition of antitoxin.

The tracing representing neutralization of the hæmolysins of the three venoms are different from the tracings of neutralization of the toxins, and approach very closely to a straight line. Still, in all instances, the determinations with great concentrations of antilysin show pronounced deviation, perhaps due to some dissociation of the toxin-antitoxin combination.

TABLE II.

TESTS OF THE ANTIVENOMOUS POWER DURING IMMUNIZATION.

The animal was bled on February 23, 1904. Guinea-pigs of 500 grm. were used for the experiment.
Serum (antivenin) 2 c.c. + crotalus venom 0.002 grm. = No symptoms.
Serum (antivenin) 2 c.c. + crotalus venom 0.004 grm. = Sick for 3 days.
Serum (antivenin) 2 c.c. + crotalus venom 0.005 grm. = Sick for 2 days; recovered.
Serum (antivenin) 2 c.c. + crotalus venom 0.0075 grm. = Death in $27^h\ 7^m$.
Serum (antivenin) 2 c.c. + crotalus venom 0.01 grm. = Death in $5^h\ 45^m$.

The animal was bled on March 23, 1904. Guinea-pigs of 400 grm. were used.
Serum 2 c.c. + crotalus venom 0.0075 grm. = No symptoms.
Serum 2 c.c. + crotalus venom 0.01 grm. = Sick for 1 day; recovered.
Serum 2 c.c. + crotalus venom 0.0125 grm. = Death in $3^h\ 35^m$.

The animal was bled on March 30 and 31, 1904. Guinea-pigs of 280 grm. were used.
Serum 2.5 c.c. + crotalus venom 0.005 grm. = No symptoms.
Serum 2.5 c.c. + crotalus venom 0.006 grm. = No symptoms.
Serum 2.5 c.c. + crotalus venom 0.0075 grm. = Death in 12^h.
Serum 2.0 c.c. + crotalus venom 0.006 grm. = Death in 16^h.
Serum 4.0 c.c. + crotalus venom 0.01 grm. = Death in 17^h.

The animal was bled on April 18, 1904. Guinea-pigs of 240 grm. were used.
Serum 2.5 c.c. + crotalus venom 0.0015 grm. = No symptoms.
Serum 2.5 c.c. + crotalus venom 0.003 grm. = Death in 12^h.

The animal was bled on May 6, 1904. Guinea-pigs of 290 grm. were used.
Serum 2.5 c.c. + crotalus venom 0.005 grm. = Slightly ill for 1 day; recovery.
Serum 2.5 c.c. + crotalus venom 0.006 grm. = Death in 12^h.

The animal was bled on May 7, 1904. Guinea-pigs of 290 grm. were used.
Serum 2.5 c.c. + crotalus venom 0.004 grm. = No symptoms.
Serum 2.5 c.c. + crotalus venom 0.005 grm. = Death in 7^h.
Serum 2.5 c.c. + crotalus venom 0.006 grm. = Death in 12^h.

The animal was bled on May 11, 1904. Guinea-pigs of 290 grm. were used.
Serum 2.5 c.c. + crotalus venom 0.003 grm. = Slightly ill; recovery.
Serum 2.5 c.c. + crotalus venom 0.004 grm. = Death in 12^h.

The milk of the same goat was taken several hours after the delivery.
Colostrum 2.5 c.c. + crotalus venom 0.003 grm. = Slightly ill; recovery.

TABLE III.

IMMUNIZATION OF A GOAT WITH THE VENOM OF WATER MOCCASIN BY SUBCUTANEOUS INJECTION.

Nov.	7, 1903,	0.0001	grm., no symptoms.
	10	0.0002	
	13	0.0004	
	16	0.0008	
	20	0.0012	
	23	0.0016	
	26	0.002	
	30	0.0025	
Dec.	4	0.003	
	9	0.004	
	12	0.004	
	16	0.005	
	21	0.008	
	26	0.01	
	31	0.015	
Jan.	2, 1904,	0.02	
	6	0.032	Small abscess at the site of injection.
	13	0.04	
	16	0.04	
	19	0.05	
	23	0.08	
	28	0.1	
Feb.	2	0.14	
	10	0.16	
	15	0.2	Ill after 24 hours.
	20		Very ill, but no local symptoms; dyspnœa, loss of appetit: and unable to stand up.
	21.		All symptoms above described increasing.
	22.		Died during the foregoing night.

TABLE IV.

IMMUNIZATION OF A GOAT WITH THE VENOM OF WATER MOCCASIN BY SUBCUTANEOUS INJECTION.

Feb.	29, 1904,	0.01	grm.
Apr.	4	0.025	No symptoms.
	9	0.04	
	15	0.07	
	19	0.09	
	23	0.12	A large abscess formed at the point of injection.
May	2	0.14	Quite well.
	6	0.18	
	9	0.2	Death after 18 hours.

TABLE V.

TOXICITY OF THE VENOM OF WATER MOCCASIN FOR A GOAT WEIGHING 27 KILO.

On April 10, 1904, at 11:43 A. M. 0.15 grm. (in 5 c.c.) of the venom of water moccasin was injected at the right side of the back, subcutaneously. After from three to four hours the animal became very inactive, but was still able to stand. It avoided lying down or moving. At the same time the whole of the injected side became intensely swollen and very painful. At the end of ten hours after the injection the animal was still able to support its body on the legs. The swelling at the site of injection became more marked and over the whole body there was swelling and œdema in varying degree, but more intense in the region of the injection. The skin around the needle puncture showed no necrosis. Death ensued at the end of twenty-two hours after injection, with symptoms of great dyspnœa.

Autopsy was performed one hour after death. There was no rigor mortis. There was enormous swelling of the entire cadaver, especially pronounced over the abdominal wall and the right back, which was very œdematous. A large quantity of bloody exudate had accumulated in the subcutaneous tissues and the exudate had a jelly-like consistence. The muscles had a dark-purple or deep blackish color, the muscles along the spinal column being darkest. The muscles were friable and very easily torn into pieces. They were infiltrated with extravasated blood corpuscles as well as with free hæmoglobin. The peritoneum, omentum, and serous membranes of the pleuræ and pericardium were free from hæmorrhage. The hæmorrhage in the subcutaneous tissue did not extend into the cervical region. Two dead fœtuses found in the uterus presented marked rigor mortis, but almost no hæmorrhage.

TABLE VI.

IMMUNIZATION WITH THE VENOM OF WATER MOCCASIN MODIFIED BY HYDROCHLORIC ACID.

Dried venom 0.5 grm.; 1 per cent. aqueous solution of hydrochloric acid 100 c.c. The mixture was kept 24 hours at 37° C.

After incubation for 24 hours at 37° C., the mixture was neutralized with hydrate of sodium, which produced a bulky precipitate of the proteids of the venom. A goat was injected subcutaneously.

Apr.	13, 1904,	0.025 grm.	
	18	0.025	
	20	0.04	
	22	0.05	
	24	0.07	
	26	0.1	
	28	0.2	
	30	0.2	
May	2	0.3	
	4	0.4	
	6	0.7	
	13		Bled.
	14	0.4	
	18	0.2	
	21	0.3	
	24	0.5	
	30		Bled (600 c.c.).

TABLE VII.

TESTS FOR ANTIVENIN DURING IMMUNIZATION.

Serum was drawn on May 13, 1904. Guinea-pigs of 290 grm. were used.
Serum 2 c.c. + moccasin venom 0.0036 grm. = Death in 7^h and 20^m.
Serum 2 c.c. + moccasin venom 0.006 grm. = Death in 2^h.
Serum 2 c.c. + moccasin venom 0.008 grm. = Death in 1^h and 50^m.
Serum 2 c.c. + moccasin venom 0.012 grm. = Death in 40^m.

Serum was drawn on May 30, 1904. Guinea-pigs of 400 grm. were used.
Serum 2 c.c. + moccasin venom 0.012 grm. = Death in 36^h.
Serum 2 c.c. + moccasin venom 0.012 grm. = Death in 1^h and 30^m.
Serum 2.5 c.c. + moccasin venom 0.006 grm. = Recovered.
Serum 2.5 c.c. + moccasin venom 0.008 grm. = Death in 10^h.
Serum 2.5 c.c. + moccasin venom 0.01 grm. = Death in 4^h 10^m.
Serum 2.5 c.c. + moccasin venom 0.012 grm. = Death in 3^h 40^m.

TABLE VIII.

RELATION BETWEEN THE BODY WEIGHT AND SUSCEPTIBILITY OF GUINEA-PIGS AFTER INTRAPERITONEAL INJECTION OF CROTALUS VENOM (NOT FILTERED).

Guinea Pigs of 500 grm.		Guinea Pigs of 250 grm.		Guinea Pigs of 125 grm.	
Venom in grm.	Result.	Venom in grm.	Result.	Venom in grm.	Result.
0.0012	+ 9^h 25^m	0.0006	+ 10^h 10^m	0.0003	+ 9^h 5^m
0.001	+ 18^h	0.0005	+ 18^h	0.00025	+ 6^h
0.0008	+ 12^h 25^m	0.0004	§ very ill	0.0002	+ 12^h
0.0008	§	0.0004	§	0.0002	+ 5^h 30^m
0.0008	§	0.0003	§	0.00015	+ 9^h
0.0008	§			0.0001	§ sick
				0.00008	§

+ = Death. § = Recovery.

TABLE IX.

DETERMINATION OF THE MINIMAL LETHAL DOSE OF CROTALUS VENOM FILTERED TROUGH THE CHAMBERLAND BOUGIE.

The stock solution was made as follows: Dried venom, 0.5 grm. and distilled water, 100 c.c. Guinea-pigs weighing 250 grm. were used and the venom was administered intraperitoneally on April 5, 1904.

Venom in grm.	Result.	Remarks.
0.0002	§	Almost no symptoms; the abdominal tension was slightly increased for one day.
0.00025	§	Ditto.
0.0003	§	Ditto.
0.00035	§	Sick for one day; the abdominal tension was much increased the first day.
0.0004	§	Ditto.
0.0005	§	Very sick for the first day; the abdominal tension was enormously increased during two days.
0.0006	§	Ditto.
0.001	§	Very sick for two days; had a very high abdominal tension; after 3 days improved rapidly.
0.0012	+ 15^h	
0.0015	+ 10^h	

+ = Death. § = Recovery.

TABLE X.

DETERMINATION OF THE MINIMAL LETHAL DOSE OF CROTALUS VENOM WITHOUT FILTRATION.

The stock solution was made as follows: Dried venom 0.5 grm.; distilled water 100 c.c. Guinea-pigs of 320 grm. were injected subcutaneously, April 21–23, 1904.

Venom in grm.	Result.	Remarks.
0.0035	+ 14 days	First day, very sick; second day, the site of injection highly swollen and the hairs came off about the site of needle puncture; the skin was softened and a small amount of a sero-sanguineous fluid discharged from the needle puncture, but the animal seemed more active than on the first day. The appetite was normal. The ulcerated area of the skin assumed greyish color (necrotic) on the third day. The animal was perfectly active until 12 days after injection, when it showed progressive emaciation, while locally there was a solid infiltrated scar. Death occurred with symptoms of marasmus.
0.005	+ 4^h	Three hours later the animal showed grave general symptoms and collapsed. Locally there was intense swelling of the tissues without any actual ulceration.
0.005	+ $3^h\,35^m$	Two hours after the injection the animal became seriously ill. Marked hæmorrhagic œdema around the site of injection, which finally spread over the entire injected side.
0.0075	§	The animal showed only slight general symptoms, while an extensive ulcer formed about the point of injection on the second day, but became dry after 7 days.
0.01	§	Showed slight stupefaction during the first day; on the second day an ulcer (3 x 4 c.c.) formed around the site of injection, but dried after 7 days.
0.015	+ $1^h\,25^m$	One hour after injection the animal fell; the local swelling was very marked. At autopsy there was very marked hæmorrhage in the muscular layers, but only moderate hæmorrhage in the mesentery.

+ = Death. § = Recovery.

TABLE XI.

TOXICITY OF CROTALUS VENOM FOR RATS (200 GRM.) AFTER INTRAPERITONEAL INJECTION.

Venom in grm.	Result.
0.0002	Well.
0.0005	Well.
0.001	Well.
0.0015	Abdominal tension increased; next day well.
0.0016	Well.
0.0018	Well.
0.002	Well.
0.002	Well.
0.002	Death 4^h.
0.0022	Well.
0.003	Well.

TABLE XII.

DETERMINATION OF THE MINIMAL LETHAL DOSE OF CROTALUS VENOM (NOT FILTERED).

Guinea-pigs weighing 270 grm. were injected intraperitoneally, April 6 to 16, 1904.

Venom in grm.	Result.	Remarks.
0.00035	§	Sick for 2 days; local infiltration disappeared after 7 days.
0.0004	§	Very sick for 2 days; local infiltration marked. 9 days later the local infiltration disappeared; after 11 days the animal was quite normal.
0.00045	+ 13 days	First day, critically ill; second day, some improvement in condition, but the whole abdominal wall was softened and formed an ulcer, 3 x 3 cm., from which a sero-sanguineous fluid discharged; third day, the surface of the ulcerated area was becoming dry and the appetite was usual; fourth day, the ulcer presented a darkish hue and the edge was demarkated. After a week the ulcer dried with a blackish crust, which gradually became smaller and fell off. The animal was nevertheless much emaciated and finally died.
0.0005	+ 17^h	Animal immediately became highly irritable and uneasy after 15 to 30 minutes; abdominal tension steadily increased. None of animals showed appetite after the injection and the hairs were ruffled. In one hour the abdominal tension became very high, and the wall became a purplish color, due to hæmorrhage. A sero-sanguineous fluid was discharged from the needle puncture. The animals became weaker gradually and finally succumbed. At autopsy there was very marked hæmorrhage in the peritoneum and viscera, and in the muscular layers of the abdominal wall.
0.00055	+ 17^h	
0.0006	+ 15^h	
0.0006	+ 21^h	
0.0006	+ 19^h	
0.001	+ 3^h 48^m	
0.001	+ 4^h 34^m	

+ = Death.
§ = Recovery.

TABLE XIII.

CROTALUS VENOM AND ANTIVENIN (I) INJECTED INTRAPERITONEALLY.

Guinea-pigs weighing 250 grm. were used. April 15, 1904. The venom was

Venom 0.06 gr. + *n* c.c. Serum.	Divided by.	Result.	Symptoms of the Animals which Finally Recovered.
0.25	8	+ 10^h	
	9	+ 15^h	
	10	§	Sick for 2 days; slight infiltration around the site of injection, lasting for 5 days.
0.5	6	+ 10^h	
	7	+ 15^h	
	8	§	Slightly ill for 1 day; slight infiltration around the needle puncture lasting for 4 days.
0.75	4	+ 12^h	
	5	+ 23^h	
	6	§	Slightly ill for 1 day.
1.0	3	+ 10^h	
	3½	+ $17^h\ 20^m$	
	4	§	Slightly ill for 1 day; slight infiltration lasting for 2 days.
	5	§	Almost no symptoms.
	6	§	Ill for several hours only.
1.25	2½	+ 15^h	Sick for 2 days; slight local infiltration lasting for 2 days.
	3	§	
1.5	1½	+ 7^h	Sick for 3 days; was very ill the first day. Local infiltration lasted for 3 days.
	2	§	
1.75	1	+ 7^h	
	1½	+ 15^h	Sick for 3 days; marked local swelling, lasting for 3 days.
	2	§	
2.0	1	+ 15^h	
	1½	§	Sick for 2 days; local infiltration for 3 days.
2.25	½	+ 17^h	
	½	+ 12^h	Very sick for 2 days; local infiltration marked and lasted for 3 days; recovery in 4 days.
	1	§	
2.5	½	§	Slightly ill for one day; recovery in 2 days.
	1	§	Almost no symptoms.
	1	§	No symptoms.
	1	§	No symptoms.

+ = Death.
§ = Recovery.

TABLE XIV.

CROTALUS VENOM-ANTIVENIN INJECTED INTO GUINEA-PIGS.

x	q Obs.	q Calc. I.	q Calc. II.
0	12	11.5	11.8
0.25	9	9	8.7
0.5	7	7	6.4
0.75	4.5	5.2	4.7
1	3.5	3.7	3.5
1.25	2.5	2.5	2.55
1.5	1.7	1.8	1.9
1.75	1.5	1.25	1.4
2	1	0.87	1.1
2.25	0.5	0.5	0.55
$p = 1$.	$K = 0.048$.	$K = 0.053$.	$n = 3/2$.

TABLE XV.

TOXICITY OF CROTALUS VENOM UPON RABBIT.

1. *Subcutaneous Injection.* (*Left Inguinal Region.*)

Venom in grm.		Weight of Animal.	Result.
0.01	25/iv	2000 grm.	+ 7 days
0.015	26/iv	2100	+ 18^h
0.02	27/iv	2100	+ $10^h\ 25^m$
0.02	16/v	1600	+ $7^h\ 40^m$
0.04	17/v	1700	+ $2^h\ 40^m$

2. *Intravenous Injection.*

0.00025	7/vi	2000 grm.	§
0.0003	7/vi	2000	+ 9 days
0.0003	2/vi	1800	+ 3 days 12^h
0.0003	24/vi	1450	+ 3 days 4^h
0.0004	30/vi	1550	§
0.0004	7/vi	2000	+ 4 days 16^h
0.0004	15/vi	2000	§
0.0005	15/vi	2000	+ 4 days 16^h
0.0005	7/vi	2000	+ 3 days
0.0005	2/vi	1750	+ $17^h\ 50^m$
0.0005	20/v	1600	+ 14^h
0.001	20/v	1600	+ 6–14^h
0.000461	11/vii	1650	§
0.0005	11/vii	1650	+ 5 days.
0.000545	11/vii	1550	+ 4 days 20^h
0.0006	11/vii	1500	+ 4 days

+ = Death.
§ = Recovery.

TABLE XVI.

Crotalus Venom-Antivenin Injected Intravenously.

Rabbits weighing about 1700 grm. were used. July 10 to 20, 1904. The venom was not filtered. Crotalus Antivenin I was used.

Venom 0.006 grm. +n c.c. Serum.	Divided by.	Result.
0	10	+ 4 days 12^h
	11	+ 4 days 20^h
	12	+ 5 days
	13	§
0.5	4	+ 4 days 12^h
	5	+ 5 days 7^h
	6	§
	7	§
1.0	2	+ 5 days 7^h
	3	+ 5 days 9^h
	4	§
1.5	1½	+ 5 days 6^h
	2	+ 5 days 9^h
	2½	§
2.0	1½	+ 4 days 12^h
	2	§
	2½	§
2.5	½	+ 4 days 12^h
	1	+ 5 days 20^h
	1½	§
3	1	§

+ = Death. § = Recovery.

TABLE XVII.

Crotalus Venom-Antivenin.

n	q Obs.
0	12
0.5	5
1.0	3
1.5	2
2.0	1.5
2.5	1.0

TABLE XVIII.

Crotalolysin.

ϵ per cent. suspension of dog's blood.
1 c.c. of 0.05 per cent. crotalus venom.
+ n c.c. of anticrotalus serum.
+ 1 — n c.c. 0.9 per cent sodium chloride solution.

n	I.	II	III.	q Obs.	q Calc.
0	0.02	0.016	0.006	100	100
0.05	0.023	0.018	0.0065	89	90.5
0.1	0.026	0.02	0.0078	77.4	81
0.15	0.028	0.023	0.0085	70.6	72

n	I.	II.	III.	q Obs.	q Calc.
0.2	0.03	0.025	0.01	63.5	62.5
0.25	0.033	0.029	0.0125	54.4	53
0.3	0.0385	0.035	0.015	45.9	44
0.35	0.052	0.043	0.019	35.8	34.5
0.4	0.078	0.065	0.026	24.4	25
0.45	0.11	0.098	0.047	15.8	16
0.5	0.275	0.24	0.12	6.3	6.5
0.55	1.5	1.15	0.35	1.5	□

TABLE XIX.

DETERMINATION OF THE MINIMAL LETHAL DOSE OF COBRA VENOM.

Stock solution was made as follows: Dried venom, 0.1 grm.; distilled water, 50 c.c.; no filtration. Guinea-pigs weighing 650 grm. were injected subcutaneously February 15, 1904.

Venom in grm.	Result.
0.00005	§
0.0001	+ 23 days
0.00015	§
0.0002	+ 36^h
0.00025	+ $18^h\ 29^m$
0.0003	+ 12^h
0.0005	+ $3^h\ 35^m$
0.00075	+ $2^h\ 3^m$
0.001	+ $3^h\ 34^m$
0.0015	+ $1^h\ 30^m$

+ = Death. § = Recovery.

TABLE XX.

DETERMINATION OF THE MINIMAL LETHAL DOSE OF COBRA VENOM.

Stock solution: Dried venom, 0.4 grm.; distilled water, 100 c.c.; filtered through a Chamberland bougie. Guinea-pigs were injected subcutaneously February 20 to March 2, 1904.

Venom in grm.	Weight of Animals in grm.	Result.
0.0001	530	§
0.00015	470	§
0.0002	550	§
0.0002	480	§
0.00025	450	§
0.0003	460	+ 13^h
0.0004	450	+ $4^h\ 39^m$
0.0005	450	+ $2^h\ 35^m$
0.001	460	+ $1^h\ 59^m$
0.0015	620	+ $1^h\ 42^m$
0.002	600	+ $1^h\ 43^m$

+ = Death. § = Recovery.

TABLE XXI.

RELATION BETWEEN DOSE AND TOXICITY.

Dose in grm.	M. l. d.	Time of Death in Hours.
0.0002	0.4	36
0.00025	0.5	18.5
* "	"	17
0.0003	0.6	12
* 0.0004	0.8	6.16
0.0005	1	3.5
* "	"	4
0.00075	1.5	2
0.001	2	3.5
* "	"	3.67
0.0015	3	1.5
* "	"	1.75
* 0.002	4	1.75

TABLE XXII.

DETERMINATION OF THE MINIMAL LETHAL DOSE OF COBRA VENOM WHEN INJECTED WITH LECITHIN.

Stock solution of cobra venom was made as follows: Dried venom, 0.4 grm.; distilled water 100 c.c.; filtered through Chamberland bougie.

The dose of lecithin injected with the venom was 1 c.c. of 1/50 N. solution. The animals used were guinea-pigs weighing 600 gram. The venom was injected subcutaneously February 24, 1904.

Venom in grm.	Result.
0.0002	§
0.0003	+ 7^h
0.0004	+ $10^h\ 30^m$
0.0005	+ $3^h\ 47^m$

+ = Death.
§ = Recovery.

TABLE XXIII.

VELOCITY OF THE REACTION BETWEEN COBRA VENOM AND CALMETTE'S ANTIVENIN.

The venom-antivenin mixture was injected intraperitoneally into guinea-pigs weighing 600 grm.

Cobra venom 0.008 grm. + Calmette's antivenin 5 c.c.

Simultaneously	1 Hour Contact at 36° C.	6 Hour Contact at 36° C.
+ 90 minutes	+ 85 minutes	+ 85 minutes

TABLE XXIV.

Cobra Venom and Calmette's Antivenin I.

Calmette's Antivenin I. 17/ii/'04.				Calmette's Antivenin I. 17/ii/'04.			
Weight of Guinea Pig in grm.	Venom 0.008 grm. + x c.c. Serum.	Divided by	Result.	Weight of Guinea Pig in grm.	Venom 0.004 grm. + x c.c. Serum.	Divided by	Remarks.
650	3	4	+ 8 min.	710	3.0	10	+ 3^h 4^m
650		7	+ 3^h 12^m	670		$13\frac{1}{2}$	+ 10^h 20^m
650		10	+ 2^h 8^m	690		20	+ 15^h 20^m
				770		28	+ 5^h 25^m
700	4	4	+ 3^h 2^m				
700		5	+ 3^h 33^m				
690		6	+ 3^h 36^m				
550		4	+ 2^h 35^m				
540	5	6	+ 2^h 50^m				
550		10	+ 7^h 50^m				
590		$13\frac{1}{2}$	§				

+ = Death.
§ = Recovery.

TABLE XXV.

Cobra Venom and Calmette's Antivenin II.

The venom solution was filtered through the Chamberland bougie before use March 9, 1904.

Calmette's Antivenin II.				
Quantity of the Antivenin Used in c.c.	Cobra Venom in grm.	Weight of Guinea Pig in grm.	Result.	Remarks.
10	0.004	450	+ 25^m	
10	0.0036	430	+ 43^m	
10	0.0032	480	+ 5^h 41^m	
10	0.0028	450	§	No general symptoms; a slight local œdema lasting for 2 days.
10	0.002	440	§	No symptoms.
One bottle (ca. 12).	0.008	510	+ 26^m	
"	0.0048	480	+ 32^m	
"	0.004	570	§	No general symptoms; marked local œdema lasting for 4 days; no loss in weight.
"	0.0032	490	§	No symptom.
Normal horse serum, 20	0.008	600	+ 15^m	
Normal horse serum, 20		600	§	Slightly inactive after 1 hour, but well after 6 hours.

+ = Death.
§ = Recovery.

TABLE XXVI.

Cobra Venom and Calmette's Antivenin. II.

The venom solution was filtered before use. The mixture of venom and antivenin was injected intraperitoneally into guinea-pigs.

Series A. Guinea Pigs of 370 grms. Calmette's Antivenin II. 11-12/iii/'04				Series B. Guinea Pigs of 450 grms. Calmette's Antivenin II. 11-12/iii/'04.			
Venom 0.0028 grm. + ½ c.c. Serum.	Divided by.	Result.	Remarks.	Venom 0.0028 grm. + ½ c.c. Serum.	Divided by.	Result.	Remarks.
2	5	+ 12h		2	4	+ 27m	
	6	+ 26h			4	+ 17h 40m	
	7	§	Slightly ill for one day.		5	+ 48h	
	8	§	Almost no symptoms.		6	§	Almost no symptoms.
4	3	+ 55m		4	2.5	+ 1h 36m	
	4	+ 36h			3	+ 14h 40m	
	5	§	Sick for one day.		4	§	Sick several hours.
	6	§	Almost no symptoms.	6	2	+ 40h	
6	2.5	+ 60m			2.5	§	Sick for two days.
	3	+ 26h 9m			3	§	Slightly ill for several hours.
	4	§	Slightly ill for one day.	8	1	+ 31m	
8	1.5	+ 28m			1.5	+ 1h 6m	
	2	§	Slightly inactive for several hours.		2	§	Sick for two days.
	2.5	§	No symptoms.		2.5	§	Slightly ill for one day.
9	1	§	Sick for one day.	10	1	§	Almost no symptoms.
10	1	§	No symptom.				

+ = Death.
§ = Recovery.

TABLE XXVII.

NEUTRALIZATION OF COBRA VENOM WITH CALMETTE'S ANTIVENIN.

The venom-antivenin mixture was injected subcutaneously into guinea-pigs weighing 250 grm.

Cobra Venom 0.003 grm. + *n* c.c. Serum.	Divided by.	Result.
0	8	+ 2^h
	9	+ $3^h\ 30^m$
	10	+ $2^h\ 30^m$
	11	+ 3^h
	12	+ 36^h
	13	§
	14	§
	15	§
1.0	5	+ 4^h
	6	+ 6^h
	7	+ 8^h
	7	+ $2^h\ 30^m$
	8	§
	8	§
2.0	3	+ 5^h
	4	+ $2^h\ 30^m$
	4	+ 4^h
	5	§
	5	§
	6	§
3.0	1	+ 36^h
	2	+ 3 days
	2	§
	2.5	§
3.5	1	+ $5^h\ 20^m$
	1	+ $3^h\ 45^m$
	1.5	§
4	1	§
	1	§

+ = Death. § = Recovery.

TABLE XXVIII.

1 c.c. 0.1 % Cobra Venom. + *n* c.c. of Antivenin I. + 1−*n* c.c. of Salt Solution (0.9 %).			1 c.c. 0.1 % Cobra Venom. + *n* c.c. of Antivenin II. + 1−*n* c.c. of Salt Solution (0.9 %).		
n	*q* obs.	*q* calc.	*n*	*q* obs.	*q* calc.
0	100	100	0	100	100
0.1	78.2	79.5	0.1	83.5	82.5
0.2	56.6	59.5	0.15	71.3	74
0.3	39.8	40.5	0.2	64.5	66
0.4	21.6	20.5	0.25	56.2	57
0.5	5.3	1	0.3	44.4	48.5
			0.4	33.3	32
			0.5	15.9	15
			0.6	3.3	0

TABLE XXIX.

DETERMINATION OF THE MINIMAL LETHAL DOSE OF WATER MOCCASIN VENOM.

Stock solution was made as follows: Dried venom, 1.0 grm.; distilled water, 100 c.c.; the solution was not filtered. The venom was injected intraperitoneally into guinea-pigs May 5, 1904.

Venom in grm.	Weight of Animal in grm.	Result.	Remarks.
0.0005	250	§	Sick for one day.
0.0008	250	§	Ditto.
0.001	250	§	Ditto.
0.001	250	§	Ditto.
0.001	250	§	Ditto.
0.0012	250	+ 7^h	Marked hæmorrhage in the abdominal cavity.
0.0012	250	+ 6^h 20^m	Ditto.
0.0014	250	+ 45^m	Very marked hæmorrhage in the abdominal cavity.
0.0024	420	+ 4^h 40^m	Ditto.
0.0024	420	+ 4^h 35^m	Ditto.
0.0024	420	+ 3^h 50^m	Ditto.
0.0024	420	+ 4^h 20^m	Ditto.

+ = Death.
§ = Recovery.

TABLE XXX.

DETERMINATION OF THE MINIMAL LETHAL DOSE OF WATER MOCCASIN VENOM.

The stock solution of venom was as follows: Dried venom, 1 grm.; distilled water, 100 c.c. The solution which was not filtered was preserved in the ice chest for 108 days. Animals were injected August 22, 1904.

Venom in grm.	Weight of Animal in grm.	Result.
0.0006	250	§
0.0008	250	§
0.001	250	§
0.001	250	§
0.0012	250	+ 5^h
0.0012	250	+ 6 days
0.0012	250	+ 5^h 20^m
0.0013	250	+ 5^h
0.0014	250	+ 4^h
0.0014	250	+ 4^h
0.0024	420	+ 4^h 40^m
0.0024	420	+ 4^h 35^m
0.0024	420	+ 3^h 50^m
0.0024	420	+ 4^h 20^m

+ = Death.
§ = Recovery.

TABLE XXXI.

Neutralization of Water Moccasin Venom.

The time of contact was 3 hours at 37° C. The animals used were guinea-pigs weighing 250 grm., and the injection was made intraperitoneally.

Venom 0.012 grm. + x c.c. of Serum.	Divided by.	Result.
2	5	+ 9^h
	5	+ 6^h
	6	+ 6^h 30^m
	6	+ 10^h 17^m
	7	
	7	
	8	
	8	
4	3	+ 3 days
	4	+ 5^h 30^m
	4	+ 16^h
	5	+ 6^h 20^m
	5	
	6	
5	3	+ 7^h 20^m
	4	
	5	
6	2	+ 5^h
	2.5	+ 16^h and 5^h
	3	
	3	
	4	
8	1.5	+ 6^h 30^m
	2	+ 18^h
	2	+ 16^h
	2.5	+ 22^h
	2.5	
9	1	+ 3^h 10^m
	2	+ 4^h 10^m
	3	+ 14^h
10	1.5	+ 5^h 30^m
	2	
	2.5	+ 6^h 40^m
	3	+ 7^h 10^m
20	2	+ 9^h
	3	+ 3^h
	4	+ 3^h
40	4	+ 3^h

TABLE XXXII.

1 c.c. of 0.05 per cent. solution of Ancistrodon piscivorus venom + 2 c.c. of antivenin + 1 — *n* c.c. of 0.9 per cent sodium chloride solution.

n	*q* Obs.	*q* Calc.
0	100	100
0.05	93	94
0.1	87	88
0.15	82	82
0.2	77	76
0.25	70	70
0.3	63	64
0.4	53	52
0.5	42	40
0.6	26	28
0.7	17	16
0.8	10.5	4
1	2	0

EXPERIMENTS ON THE LEUKOLYTIC ACTION OF THE BLOOD SERUM OF CASES OF LEUKÆMIA TREATED WITH X-RAY AND THE INJECTION OF HUMAN LEUKOLYTIC SERUM IN A CASE OF LEUKÆMIA.

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Contents.

I. The effect of the blood serum of leukæmic patients exposed to X-ray upon leukocytes of injected animals.
II. The effect of X-ray on phagocytosis.
III. The effect of the serum of leukæmic patients exposed to X-ray on the human leukocytes and erythrocytes, as observed in the hanging drop.
IV. The effect of heat on leukolytic blood.
V. The effect of X-ray upon the blood of leukæmic patients in vitro.
VI. The effect of X-ray upon the blood of leukæmic patients in vivo.
VII. The injection of a strong leukolytic serum from a leukæmic patient exposed to X-ray into an untreated case of leukæmia.
VIII. The nature of leukolysin and the manner of its production.

Since the discovery of the remarkable effects of X-ray upon the blood, spleen, and glands in leukæmia, numerous attempts have been made to explain its action. Experiments for this purpose have been fruitful, but they have been, for the most part, restricted to animals. Human experiments are obviously preferable to animal ones, whenever they can be carried out, for the reaction of a healthy animal to X-ray is not necessarily the same as that of man in disease. In such an investigation as the present one it is desirable to work with human material, since leukæmia is rarely found in animals, and it has not been produced artificially.

For the purpose of observation we were fortunate in having at our disposal four cases of leukæmia, three of which were of the lymphatic and one of the splenomyelogenous variety. These cases exhibited many phases of reaction to X-ray treatment. One patient gave a favorable therapeutic result; another, a fair result; a third,

a slight improvement only; a fourth, a good result, followed by a relapse.

Finally, we were able to inject the blood serum of the patient who was most improved directly into a patient who was not under the influence of the X-ray.

I. THE EFFECT OF THE BLOOD SERUM OF LEUKÆMIC PATIENTS EXPOSED TO X-RAY UPON THE LEUKOCYTES OF INJECTED ANIMALS.

It was first pointed out by Heineke[1] that lymphoid cells are very susceptible to X-ray. Linser and Helber[2] after numerous animal experiments concluded that X-ray destroys not only the lymphoid cells of the glands and spleen, but also the leukocytes of the circulating blood, attacking first the young mononuclear forms. They found, furthermore, that following the treatment a new substance, a leukotoxin, was produced in the blood, that when injected into animals destroyed the circulating leucocytes, and when added to animal exudates containing leukocytes, caused loss of motion and degeneration of the cells.

A series of animal injections was carried out by us on similar lines to those of Linser and Helber, except that we used human instead of animal serum. The leukocytes of the animals treated were counted before the injection, and at intervals of about two, five, eight, twenty-four, and forty-eight hours afterward. The maximum effect was usually seen in twenty-four hours, but was sometimes delayed until forty-eight hours. The count made at the expiration of the first twenty-four hour period was used for comparison with the normal.

A. *Normal Human Serum.*

Experiment 1.—Blood drawn from the veins of a healthy man (C.) with Luer syringe was defibrinated and centrifuged under aseptic conditions. 3 c.c. of serum injected into rabbits caused in twenty-four hours an increase of about 10 per cent. in the number of leukocytes.

Experiment 2.—5 c.c. of the same serum caused in the same time an increase of 12 per cent. in the number of leukocytes.

Experiment 3.—3 c.c. of serum taken from a healthy man (S.) injected into

[1] Heineke, *Münch. med. Woch.*, 1904, li, 785.

[2] Linser and Helber, *Cong. für innere med.*, 1905, xxii, 143.

rabbits caused in twenty-four hours an increase of 5 per cent. in the number of leukocytes.

Experiment 4.—3 c.c. of the same serum injected into guinea-pigs caused in twenty-four hours a rise of 15 per cent. in the number of leukocytes.

B. *Leukæmic Serum.*

Experiment 5.—The blood was obtained from a case of lymphatic leukæmia (Eich.) before the X-ray treatment was begun. 2.5 c.c. of the serum were injected into a rabbit and caused in twenty-four hours a rise of 25 per cent. in the number of leukocytes.

Experiment 6.—2.5 c.c. of the same serum injected into a guinea-pig was followed by a rise of 5 per cent. in the number of leukocytes.

Experiment 7.—The blood was obtained from a case of splenomyelogenous leukæmia (Butcher). 2.5 per cent. of the serum injected into a rabbit was followed in twenty-four hours by a rise of 50 per cent. in the number of leukocytes.

Experiment 8.—2.5 c.c. of the serum injected into a guinea-pig was followed in twenty-four hours by a rise of 20 per cent. in the number of leukocytes.

Experiment 9.—The blood was obtained from a case of lymphatic leukæmia (Knox) which had not been under treatment for three months. 3 c.c. of the serum injected into a rabbit was followed in twenty-four hours by a rise of 56 per cent. in the number of leukocytes.

Experiment 10.—2.5 c.c. of the same serum injected into a guinea-pig was followed in twenty-four hours by a rise of 20 per cent. in the number of leukocytes.

C. *Leukæmic Serum from a Patient under X-ray Treatment.*

Experiment 13.—The blood was obtained from a case of lymphatic leukæmia (Fifield) which had been under regular X-ray treatment for eighteen months, and which showed, at the time of bleeding, glands and spleen of almost normal size, and a leukocyte count of less than 10,000 per c. mm.

2.5 c.c. of the serum injected into a rabbit was followed in twenty-four hours by a decrease of 35 per cent. in the number of leukocytes.

Experiment 14.—2.5 c.c. of the same serum injected into a guinea-pig was followed in twenty-four hours by a decrease of 60 per cent. in the number of leukocytes.

Experiment 15.—The blood was obtained from a case of lymphatic leukæmia (Knox) under regular X-ray treatment for two months, which had been much improved, but in which the glands and spleen were still enlarged, and the leukocytes varied from 30,000 to 50,000 per c. mm. 3 c.c. of the serum injected into a rabbit was followed by a fall of 40 per cent. in the number of leukocytes.

Experiment 16.—3 c.c. of the same serum injected into a guinea-pig was followed by a decrease of 12 per cent. in the number of leukocytes.

Experiment 17.—The blood was obtained from a case of splenomyelogenous leukemia (Butcher) which had been under regular X-ray treatment for three months. The spleen was about one-half as large as at the beginning of the treatment, and the leukocytes varied from 40,000 to 60,000 per c. mm. 2.5 c.c.

of the serum injected into a rabbit was followed in twenty-four hours by a fall of 20 per cent. in the number of leukocytes.

Experiment 18.—2.5 c.c. of the same serum injected into a guinea-pig was followed in twenty-four hours by a fall of 10 per cent. in the number of leukocytes.

Summary.—The injection of normal human serum in rabbits and guinea-pigs is followed in twenty-four hours by a slight increase in the number of leukocytes.

The injection of serum from cases of leukæmia is followed by a more marked rise in the number of leukocytes.

The injection of serum from cases of leukæmia which have been treated by X-ray causes within twenty-four hours a distinct fall in the number of leukocytes. The fall is greatest with the serum of patients who have shown the most decided improvement under X-ray treatment. The fall is least with the serum of patients who have received the slightest benefit from the treatment. We are, therefore, warranted in concluding that the amount of leukolytic substance present in the blood of these cases probably varies directly with the degree of clinical improvement observed in the cases.

Differential leukocyte counts of the blood of animals injected with the serum showed that the mononuclear cells are more influenced than the polynuclear ones. Hence the leukolytic action is, to a certain extent, selective.

II. THE EFFECT OF X-RAY ON PHAGOCYTOSIS.

In these experiments on phagocytosis the following method was employed: The blood to be tested was defibrinated and placed in two tubes, one of which was exposed to the X-ray for twenty minutes, while the other was kept as a control. To both of these was added a definite amount of a twenty-four hour culture of anthrax bacillus or staphylococcus, after which the tubes were well shaken. At intervals of thirty minutes, one hour, and at intervals of from two to four hours, a drop was taken from each tube, spread on a slide with rice paper and stained with Leischman's stain. A large number of leukocytes, never less than fifty, was then counted, and the percentage of leukocytes containing bacteria was noted. By comparing these results with those observed in the controls one could say whether X-ray treatment increased or diminished phagocytosis.

The blood of two healthy men, and of one case of splenomyelogenous and one of lymphatic leukæmia, was treated in this way. As it was thought that the glass of the test tube might cut off many of the X-rays, most of the experiments were repeated, using a shallow vessel with a cotton covering for the blood. In this case the rays were applied from above. The results were practically identical with the test tube experiments.

In normal blood and in lymphatic leukæmia the X-ray did not materially alter the number of phagocytes or the number of bacteria in the leukocytes. In splenomyelogenous leukæmia, phagocytosis was slightly impaired. These results are easily interpreted when one considers that the polynuclear cells are the only leukocytes capable of phagocytosis, whereas the cells influenced chiefly by the X-ray are the mononuclear ones and the myelocytes, the polynuclear cells being most resistant. In other words, the X-ray may destroy large number of myelocytes or mononuclear cells without materially affecting the phagocytic power of the polynuclear cells.

In the light of these observations it is difficult to understand the assertion of Quadrone[3] that X-ray, by stimulating the phagocytic power of the leukocytes, increases the resistance of animals to disease. He injected various pathogenic bacteria into mice and guinea-pigs, and found that the animals which had been exposed several times to X-ray resisted the infection better than the control animals.

III. THE EFFECT OF THE SERUM OF LEUKÆMIC PATIENTS EXPOSED TO X-RAY ON THE HUMAN LEUKOCYTES AND ERYTHROCYTES, AS OBSERVED IN THE HANGING DROP.

The technique was as follows: The leukocytes used in each experiment were obtained by centrifuging a tube of freshly defibrinated blood; from the top of this tube a platinum loop was filled and placed on a cover-glass. This drop was made up of a large number of leukocytes mixed with a good many red corpuscles. A loop of the serum which was to be tested was added to the drop, and the two were thoroughly mixed on the cover-glass. The cover was placed on a hollow-ground slide and the edges were rimmed with vaselin to prevent drying. The effect of the serum on both the

[3] Quadrone, *Cent. für innere Med.*, 1905, xxvi, 521.

leukocytes and erythrocytes was observed and compared with the controls.

All of the experiments in the table were repeated with leukocytes which had been washed free of red corpuscles and serum with normal salt solution. The behavior of the leukocytes was essentially the same as in the unwashed specimens.

It will be noted that the three cases of leukæmia which were employed represent entirely different clinical reactions to the X-ray treatment. The first (Eich) responded only slightly; the second (Butcher) improved considerably; and the third (Fifield) was improved to the extent of being quite free from subjective or objective symptoms of the disease. Experiments 14 and 15 were intended to show whether the serum of Butcher, which responded only partially to X-ray, had any neutralizing effect upon the strong leukolytic serum of Fifield. The results on this point were not conclusive.

Summary.—1. Leukæmic serum which has not been treated has no leukolytic action on normal blood nor on other leukæmic blood. It has little or no agglutinating action on normal erythrocytes or on the erythrocytes of other leukæmias.

2. The serum of cases of leukæmia which has been treated with X-ray has a decided leukolytic action on normal blood and on other leukæmic blood, and this effect is in direct ratio to the clinical reaction of the patient to the X-ray, as shown by symptomatic improvement.

3. The leukolytic action is selective, showing a marked preference for the mononuclear cells and the myelocytes.

4. The serum of leukæmia which has been treated with X-ray agglutinates the erythrocytes of normal blood and other leukæmic blood. The degree of agglutination corresponds roughly to the degree of leukolysis present.

IV. THE EFFECT OF HEAT ON LEUKOLYTIC BLOOD.

According to Linser and Helber, the leukolytic substance is destroyed by heating the serum to 55° or 60° C. Experiments 9 and 10 with the hanging drop (see Table) and the experiments repeated with the washed leukocytes showed only an impairment of leukolytic action of the serum of leukæmia treated with X-ray after

No. Exp.	Mixture of One Loop Each.		Effect Upon.	
	Leukocytes with Many Red Cells.	Serum.	Red Cells.	Leukocytes.
1	Normal (Coles).	0.9 % NaCl sol.	No rouleaux; no clumps.	No swelling in 60°; no fragmentation in 20°.
2	Normal (Coles).	Normal (Thomas).	Rouleaux formation; no clumps.	Swelling in 6°; no fragmentation in 18°.
3	Normal (Thomas).	Normal (Coles).	Moderate rouleaux; no clumps.	Only swelling in 10°; no fragmentation in 20°.
4	Normal (Thomas).	Lymph. leuk. (Eich)[4] before *x*-ray.	No tendency to clump.	No swelling in 4°; no fragmentation in 20°.
5	Normal (Thomas).	Same after *x*-ray in vivo.	Moderate degree of clumping.	Swelling in 4°; fragmentation slight in 10°.
6	Normal (Thomas).	Same after *x*-ray in vivo and also in vitro.	Same degree of clumping.	Swelling in 4°; fragmentation moderate in 10°.
7	Normal (Thomas).	Sp. myel. leuk. (Butcher) *x*-ray in vivo.[5]	Moderate amount of clumping.	Swelling in 3°; fragmentation in 8°.
8	Normal (Thomas).	Same *x*-ray in vivo and also in vitro.	Same amount of clumping.	Swelling in 3°; fragmentation more marked in 8°.
9	Normal (Thomas).	Same heated 60° C. for 30′.	Same as above.	Swelling and fragmentation slight; less marked.
10	Normal (Thomas).	Lymph. leuk. (Fifield) *x*-ray in vivo.[6]	Clumping very marked and immediate.	Swelling and clearing in 2°; fragmentation in 4°, very marked.
11	Normal (Thomas).	Same heated 60° C. for 30′.	Same.	Less marked, but still considerable.
12	Sp. myel. leuk. (Butcher).	Normal (Coles).	No clumps; rouleaux present.	No swelling in 6°; no fragmentation in 20°.
13	Lymph. leuk. (Fifield).	Normal (Coles).	No clumps; rouleaux present.	No swelling in 6°; no swelling in 20°.
14	Normal (Thomas).	1 loop (Butcher) + 1 loop (Fifield).	Clumping marked.	Swelling and fragmentation less marked than X; swelling and fragmentation same as VII.
15	Normal (Thomas).	2 loops (Butcher) + 1 loop (Fifield).	Clumping fairly marked.	Swelling and fragmentation less marked than XIV.
16	Lymph. leuk. (Fifield).	Sp. myel. leuk. (Butcher).	Moderate clumping.	Swelling in 3–4°; fragmentation in 6°, especially myelocytes.
17	Sp. myel. leuk. (Butcher).	Lymph. leuk. (Fifield).	Clumping very marked and immediate.	Swelling in 2–3°; fragmentation in 3–4°, especially myelocytes.
18	Sp. myel. leuk. (Butcher).	Same heated 60° C. for 30′.	Same.	Considerably less swelling and fragmentation.

[4] Eich—lymphatic leukæmia; only slight reaction to X-ray; leukocytes = 300,000.
[5] Butcher—splenomyelogenous leukæmia; considerable improvement under X-ray; leukocytes = 40,000.
[6] Fifield—lymphatic leukæmia; very marked improvement under X-ray; leukocytes = 8,000.

being heated for thirty minutes to 60° C. The agglutinating effect of the serum upon the erythrocytes was not altered by the heat. However, we consider these experiments inconclusive as to whether the leukolytic substance is thermolabile or thermostabile. For, if the leukolytic substance is made up of amboceptor and complement, and the complement is destroyed by heat, fresh complement is supplied by the test leukocytes in the hanging drop. The same objection exists to injecting the serum, after heating, into animals, for fresh complement may be furnished by the blood of the animals.

We attempted to obtain anticomplement, which, by replacing the destroyed complement, would render the leukolysin inert; but the animal injected for this purpose died during the process of immunization.

V. THE EFFECT OF X-RAY UPON THE BLOOD OF LEUKÆMIC PATIENTS IN VITRO.

A. *Normal Blood.*

Experiment 1.—2 c.c. of normal defibrinated blood (S.) were exposed for twenty minutes to X-ray in a test tube, at a distance of four inches. Under the microscope, no swelling nor fragmentation of the leukocytes were seen. When a loop of serum was added to a loop of normal leukocytes (C.) and examined in the hanging drop, within ten hours no leukolysis was observed.

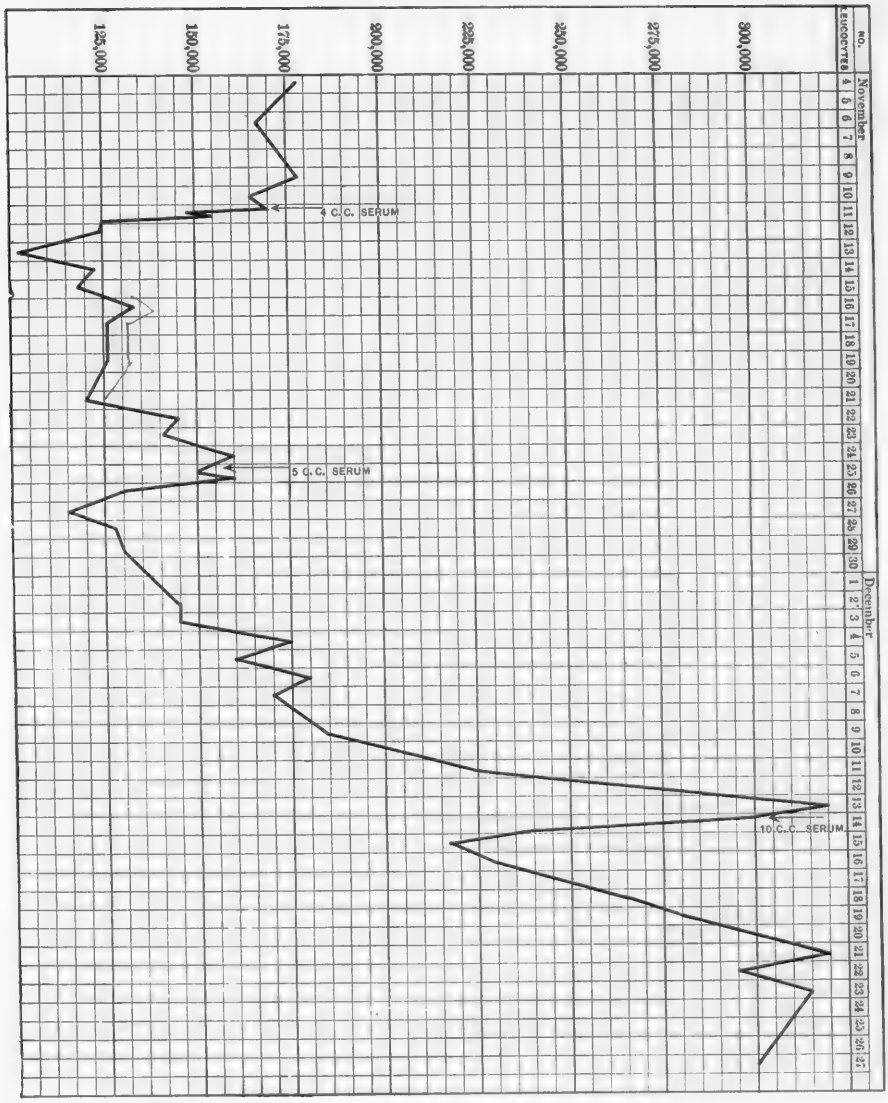
Experiment 2.—The above experiment was repeated, using normal blood (C.) for the X-ray exposure, and normal leukocytes (S.) for the test leukocytes. The results were identical.

B. *Leukæmic Blood.*

Experiment 3.—In a case of lymphatic leukæmia (Eich.) which had been treated four times with X-ray, the serum of which is slightly leukolytic, the leukocytes numbered about 300,000. After a thirty-minute exposure in a test tube to X-ray, the leukolytic action of the serum was increased, and the leukocytes showed, in the next twelve hours, more swelling and fragmentation than those in the control tubes.

Experiment 4.—A case of splenomyelogenous leukæmia (Butcher) which was under X-ray treatment most of the time for twelve months, showed only moderate reaction. The leukocytes numbered about 50,000 and the serum was moderately leukolytic. After X-ray treatment for thirty minutes, in the course of the next ten hours the leukocytes gave evidence of greater disintegration than the control leukocytes. The myelocytes were the most affected, and the serum became more leukolytic.

Experiment 5.—A case of lymphatic leukæmia (Knox) at first improved under the X-ray treatment and then relapsed. The leukocytes numbered about


NO. LEUCOCYTES
125,000
150,000
175,000
200,000
225,000
250,000
275,000
300,000
November
December
4 C.C. SERUM
5 C.C. SERUM
10 C.C. SERUM

40,000. Before treatment, the serum was not leukolytic, and after being treated in a tube for thirty minutes, it was not leukolytic to any extent. The leukocytes in the exposed tube showed a slight swelling and an earlier fragmentation than those in the control tube.

Summary.—Single direct exposure to X-ray of normal blood in the test tube causes no appreciable destruction of the leukocytes, nor is the serum leukolytic to any extent. Direct exposure of leukæmic blood produces varying degrees of fragmentation of the treated leukocytes and imparts, in some cases, a leukolytic property to the serum. The development of leukolytic substance seems to be greatest when the leukocytes are most abundant, and slight or absent when but few leukocytes are present. It is probable that in normal blood too few leukocytes are present to produce any considerable amount of leukolysin.

VI. THE EFFECT OF X-RAY UPON THE BLOOD OF LEUKÆMIC PATIENTS IN VIVO.

Single Exposure.

Experiment 1.—A patient with splenomyelogenous leukæmia (Butcher) had taken no X-ray treatment for twelve weeks. The leukocytes numbered about 36,100. After vigorous treatment with X-ray for thirty minutes over the spleen and liver, three hours later, the leukocytes numbered about 29,800; six hours later, they numbered 26,000; twelve hours later, 28,000; thirty hours later, 26,000. A differential count made it evident that the decrease had been chiefly in the myelocytes. Before the X-ray treatment, about 5 per cent. of the leukocytes were fragmented; twenty-four hours after treatment nearly 20 per cent. were fragmented.

Experiment 2.—A case of lymphatic leukæmia (Knox) had been given no X-ray treatment for over a month. The glands of the neck and spleen were then exposed to the X-ray for thirty minutes. Before the treatment, the leukocytes numbered about 106,000; six hours after, they numbered 112,000; twenty-four hours after 94,000; and forty hours after, 84,000.

In a drop of blood taken forty hours after the X-ray exposure, a larger number of free nuclei were to be seen than in the blood taken before the treatment was administered.

Repeated Exposures.

Four cases of chronic lymphatic leukæmia were exposed to X-ray over periods varying from four months to two years. In every case there was a steady fall in the number of leukocytes, until the count was normal or nearly so. The rate of decrease depended upon the frequency and length of duration of the exposures, but it also varied

with the individual. When the patient suffered a relapse the response to the treatment was slower, and after a time failed altogether. The mononuclear cells suffered relatively far more than the other cell types. Two cases of chronic splenomyelogenous leukæmia reacted to X-ray treatment in a similar way, with the exception that the myelocytes were destroyed more than any other cells.

This selective action of the X-ray in animals was first demonstrated by Linser and Helber.[7] Bozzolo[8] states that several hours after treatment the leukocyte count in splenomyelogenous leukæmia rises rapidly and then sinks again. Other observers, including Hoffman,[9] have failed to find such an increase. After long continued treatment, the X-ray may lose its effect upon the glands, spleen and blood. This indicates that the individual may acquire immunity to the X-ray.

VII. THE INJECTION OF A STRONG LEUKOLYTIC SERUM FROM A LEUKÆMIC PATIENT EXPOSED TO X-RAY INTO AN UNTREATED CASE OF LEUKÆMIA.

The serum in this case was obtained from a patient suffering from lymphatic leukæmia (Fifield), who had been under X-ray treatment at regular intervals for nearly two years. The serum, which had been tested upon leukocytes in the hanging drop and by means of animal injections, was found to be leukolytic to a high degree. The subject for the injection was a case of lymphatic leukæmia, which, for three months, had not been treated in any way and had been getting steadily worse, as was indicated by the increasing number of leukocytes, and the enlarging glands and spleen. The blood was obtained under aseptic precautions, and after being defibrinated and centrifuged the serum was drawn off and injected into the abdominal wall of the patient.

The accompanying chart illustrates the rapid fall in the number of leukocytes after each injection of the serum. The decrease was greatest at the expiration of the twenty-four to forty-eight hours following the injection. After the first injection of 4 c.c. of the

[7] *Loc. cit.*

[8] Bozzolo, abstract from *Reforma Medica*, 1905.

[9] Hoffman, *Cong. f. innere Med.*, 1905, xxii, 125.

serum the leukocytes decreased in forty-eight hours by 64,000. After the second injection of 5 c.c. of the serum, in forty-eight hours the leukocytes decreased by 34,000. After the third injection of 10 c.c. of the serum, in twenty-four hours the leukocytes decreased by 82,000. It will be observed that the leukocytes attained the original count or a higher one in periods of thirteen, nine, and six days, respectively, after the injection. Although the amount of serum injected was increased, the effect was more transitory each time. Here again is indicated a partial immunity to the leukolytic serum similar to the immunity to the X-ray itself, which may be acquired by a patient. The destruction of the leukocytes was selective, the mono-nuclear cells being the ones chiefly attacked.

In this connection may be cited the interesting experiments of Flexner[10] and of Bunting[11] upon the influence of myelotoxic and lymphotoxic sera upon the blood and blood-forming organs. These sera cause first a leukopenia, then a leukocytosis, and, finally, a return to the normal. The lymphotoxic serum caused, at first, a lymphopenia and, afterwards, a lymphocytosis, and its injection was followed by hypertrophy of the lymphatic glands.

VIII. THE NATURE OF LEUKOLYSIN AND THE MANNER OF ITS PRODUCTION.

Leukolysin is probably formed by the destruction of the leukocytes by the X-ray. This action is seen in the experiments with the leukæmic blood in test tubes exposed to X-ray, where both the fragmentation of the leukocytes and the formation of the leukolytic serum can be demonstrated. In the living subject the leukocytes in the circulating blood and the spleen may alone be the source of leukolysin; or this function may be shared by the lymphoid cells of the glands and spleen. The latter seems to us the more probable, because the formation of leukolysin and evidences of leukocyte destruction are much more marked after a given exposure of the spleen and glands to X-ray than after an exposure of the blood in the test tube. Moreover, we know that lymphoid cells, like the leukocytes, are susceptible to X-ray.

[10] Flexner, *Univ. of Penna. Med. Bulletin*, 1902, xv, 287.
[11] Bunting, *Ibid.*, 1903, xvi, 200.

The nature of this leukolysin remains in doubt. It may be an amboceptor-complement complex or a true toxine.

General Conclusions.—1. The X-ray produces in leukæmia a disintegration of the leukocytes, affecting especially the young forms, viz., the myelocytes and the non-granular mononuclear cells. A similar action, but one of less degree, takes place in leukæmic blood exposed to X-ray in vitro.

2. The serum of a leukæmic patient who has improved under the X-ray treatment, when injected into animals, causes leukopenia; when added in the hanging drop to the leukocytes of another individual it disintegrates the cells. This leukolytic action is selective, destroying first the mononuclear cells. The strength of the leukolytic action seems to be proportional to the degree of clinical improvement of the patient under the X-ray treatment.

3. The serum of a case of leukæmia which has been exposed to X-ray has a marked agglutinating action on normal red corpuscles and on other corpuscles. The degree of agglutination varies roughly with the degree of leukolysis present.

4. X-ray treatment of normal or leukæmic blood in vitro does not materially alter the phagocytic power of the leukocytes.

5. The injection of a strong leukolytic serum from a patient suffering from lymphatic leukæmia under X-ray treatment into another individual with lymphatic leukæmia, not under the treatment, caused a decided and rapid fall in the number of leukocytes. The mononuclear cells were principally affected. With repeated injections a partial immunity to the serum was established.

We take this opportunity of acknowledging our indebtedness to Drs. Billings, Bevan and Herrick for clinical material, and to thank especially Drs. Hektoen and Ricketts for their many helpful suggestions.[12]

[12] Read at the meeting of the Association of American Physicians, Washington, D. C., May 15–16, 1906.

OBSERVATIONS ON THE CYTOLOGY OF MULTIPLE NON-INFLAMMATORY NECROSIS OF THE LIVER AND ON CERTAIN RELATED DEGENERATIVE CHANGES IN CELLS.[1]

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There has been described recently from this laboratory[2] an independent affection of the liver characterized anatomically by "multiple, irregular, circumscribed solution of liver cells without parenchymatous degeneration or coagulation necrosis, and associated with a corresponding lobular blood and bile stasis in the affected areas. It appeared that the protoplasm and later the nucleus of the cells simply wasted and dissolved, leaving a more or less well preserved reticulum with stagnant blood and bile." The condition has been observed here six times (two unpublished cases) and seems to be characterized clinically by jaundice and mental symptoms, usually delirium, followed by a rapidly fatal issue. To the lesion as a whole Oertel has applied the term "multiple non-inflammatory necrosis of the liver with jaundice." The cellular changes he designates "cytolysis."

While several observers, notably Flexner in this country and Fr. Muller in Germany, correlating chemical with morphological findings, have sought to explain certain pathological processes on the basis of the property of certain cells to elaborate digestive ferments, practically no work has been done on the structural changes brought about in the tissues by the action of such substances. Hauser[3] in 1886 kept small pieces of liver under aseptic conditions for varying lengths of time. The specimens gradually softened.

[1] This study was aided by an appropriation from the Rockefeller Institute for Medical Research.

[2] *Jour. Med. Research,* 1904, xii, 75. *Jour. Exper. Med.,* 1906, viii, 103. *Collected Proc. N. Y. Path. Soc.,* new series, 1905–6, v, 143.

[3] *Arch. für exper. Path. v. Pharm.,* 1886, xx, 162.

Microscopically the cell form was found to be preserved to a late date, the nuclei were degenerate and the cytoplasm appeared granular. Waldvogel[4] recently repeated these experiments with like results. Both these observers, however, were working on problems which did not intimately concern the morphology of autolysis and consequently their description of the cellular changes is lacking in detail.

The present communication has to do, first, with a detailed description of the cell changes in multiple non-inflammatory necrosis of the liver and with related cell changes in other conditions, and, second, with the description of a variety of cell degeneration likewise affecting the liver and, I believe, generically allied to that of Oertel's lesion but presenting distinctly different characteristics, both general and cytological.

In multiple non-inflammatory necrosis the individual liver cells are large and, although irregular in outline, the various forms are well preserved. The nuclei are relatively large and occupy, as a rule, a central position in the cell body, being pushed aside only in those instances in which the cytoplasm has been the seat of fat deposit. The cytoplasm appears pale and washed out and stains poorly. The nucleus, relatively much richer in substance, stains correspondingly deeply; its shape for the most part is well preserved and the nuclear membrane appears to be intact and stains deeply, while immediately within the membrane are numerous large and small, deeply staining granules which follow the circumference of the nucleus. The center of the nucleus is pale and turbid and not infrequently is traversed by fine chromatin bands which appear to radiate from the relatively large, rounded, deeply staining nucleolus or nucleoli. In those cells in which the nucleus appears only slightly disintegrated the cytoplasm is correspondingly well preserved.

The change in the cytoplasm appears primarily to be a simple solution of the hyaloplasm with retention of the spongioplastic network, giving the cell a finely honeycombed appearance. In places the meshes of the spongioplasm are partly occupied by minute bile particles; in other places certain alveoli seem to have coalesced to

[4] *Virchow's Arch.*, 1904, clxxvii, 1; *Deut. Arch. für klin. Med.*, 1905, lxxxii, 437.

form a large vacuole for the reception of aggregations of such particles. In other places the cytoplasm shows large vacuoles which, in fresh sections stained in Sudan III, reveal the presence of neutral fats.

A further step in the destruction of the cell after the hyaloplasm has been dissolved out is represented by shrinkage of the spongioplasm, followed by enlargement and distortion of the cell body and partial or complete nuclear destruction. The fine spongioplastic network may be retained in places and lost in others. In the end all that remains is a wrinkled but otherwise intact cell membrane enclosing, in some instances, a few coarse, irregularly distributed reticular fragments and occasional fat droplets or aggregations of bile.

It is found, however, that cell changes simulating those just described are not confined to multiple non-inflammatory necrosis of the liver. In the exudate in resolving pneumonia, in the cells that lie free in the alveoli in chronic cyanosis of the lungs, and in cerebral softening, similar structural changes are apparent, brought about, it is believed, by solvent action.

The solution of the red cells can be traced both in the capillaries and smaller vessels and in the alveoli, especially in chronic cyanosis of the lungs. The first change appears to consist of partial solution of the hæmoglobin manifested by a diminution of the avidity with which the cell takes up the acid stain. The corpuscle stains a dull pink or dirty yellow and becomes opaquely granular. From this point the staining qualities can be traced through certain gradations in color until finally the cell stains very faintly or not at all, appearing as a definitely rounded body with an intact cell membrane which encloses a pinkish or bluish or grayish opaquely granular material, the cell varying in diameter from five to twelve microns or even more. Finally the altered cell substance is completely dissolved out and all that remains is the cell membrane. This structure frequently retains its rounded shape, but occasionally appears wrinkled —the "shadow" or "ghost" cell. In the pulmonary alveolus the pale, definitely rounded, non-nucleated cells constitute a characteristic feature, the peculiar constitution of the cytoplasm being very striking. It is apparent, also, that after being extruded into the

alveolus the corpuscle often increases in size, an effect due, no doubt, to a variety of factors.

In the white cells certain individual morphological variations are presented. The leucocytes earliest affected are comparable to the large lymphocytes; the polymorphonuclear cells, while not infrequently involved, appear to be relatively immune or at least more resistant. The first visible change in the leucocytes consists of swelling of the cytoplasm, which becomes cloudy and stains a dull red with eosin. It then appears to lose entirely or almost entirely its affinity for the acid dye, staining faintly blue or pink. The cells become more or less irregular both in shape and size, although the continuity of the enclosing membrane is practically always preserved. At this stage the cytoplasm shows a distinct reticular arrangement with the formation of empty alveoli. The nuclei display various degrees of disintegration and are much distorted. The further destruction of the cell may be accomplished, however, in different ways and the various steps cannot well be followed. Sometimes the cytoplasm at the periphery is lost, a few irregular clumps being arranged around the rapidly disintegrating nucleus. Or the nucleus may be found lying next the cell membrane, where frequently it becomes flattened or cresentic in outline, while the cell body is made up of irregularly scattered reticular remnants. Finally the nucleus is lost and the cell appears either as an irregularly rounded body enclosing a few reticular fragments or as a mere shell.

In chronic encephalitis with extensive softening the leucocytes in the softened areas and, to a less extent, those in the better preserved parts of the brain, show all varieties of the degenerative changes just described that lead to the exposure of the cell reticulum. In addition there are to be seen numbers of large, very pale leucocytes whose outline is irregular but whose cell membrane is preserved. The cytoplasm appears to be made up of opaque, structureless material and the nucleus is markedly degenerate. In some of these cells the nucleus has disappeared and the cell membrane encloses opaque, amorphous material only, which may be scattered evenly over the surface or arranged irregularly in clumps. In still other cells the membrane encloses nuclear fragments and small

collections of debris. In the end the cell membrane alone remains.

This peculiar appearance of the cell may be likened to that of the cytolyzed red cells already described, or more aptly still to certain structural changes in the liver cells to be described later.

Related changes can be followed in the alveolar epithelium in pneumonia and in chronic cyanosis of the lungs. These cells, as they lie attached to or partially detached from the alveolar wall, appear as large, palely staining, flattened or elongated bodies with pale, rounded or oval nuclei which enclose rather deeply staining nucleoli. The cytoplasm presents a distinct reticulum and the hyaloplasm is not visible. After the cell is completely detached and lies free in the alveolus it often becomes swollen and irregular in shape, but retains otherwise the general characteristics just described. Its identification in this position can usually be determined by the fact that its nucleus encloses a nucleolus, by the shape of the cell and by the occurrence of large, golden brown pigment particles, although similar pigment masses now and then can be seen lying upon or within cytolyzing leucocytes.

In connection with the general question of cytolysis I wish to draw attention to a form of cell degeneration occurring in the liver, for which, assuming that the morphological interpretation is correct, because of the character of the cellular changes presented, I would suggest the term "cytolysis granulosa." Since the condition was first detected I have looked for it in about two hundred cases and have been able to find it present seven times as a widely spread, diffuse affection and several times as a more restricted process. Neither the clinical histories of the respective patients nor the microscopical findings in the different organs throw much light on the changes in the liver cells. Three patients presented well-marked anatomical evidences of syphilis. The gross appearance of the liver was not regarded in any case as at all remarkable.

In three instances the autopsy was performed within four or five hours after death. Specimens were preserved in 5 per cent. formalin solution and examined in frozen or celloidin and paraffin sections. The change appears to show to best advantage when sections are deeply stained in Böhmer's hæmotoxylin and examined under the oil immersion lens.

In this condition the liver cells appear for the most part to be arranged in definite rows, closely packed together; the cell outline is unusually distinct and the normal shape almost always is retained. The intercolumnar vascular and lymphatic channels are wide. Under the oil immersion lens the cells everywhere appear pale and of average size. The cell membrane stains deeply and stands out prominently against the pale cytoplasm. The nucleus almost uniformly is enlarged out of proportion to the size of the cell as a whole and is usually rounded, occasionally indented, in most cells lying in the center, in some being pushed to one side. In what seems to be the initial step in the process the cytoplasm looks pale and made up of turbid, granular looking, structureless material evenly but thinly distributed over the surface of the cell, and composed apparently of a combination of altered hyaloplasm and broken-down reticulum. The nucleus now shows beginning central turbidity with small chromatin granules arranged peripherally. A nucleolus—sometimes more than one—may be present. In cells still more badly damaged this hazy, dirty looking material is arranged in irregularly scattered clumps, while between the clumps are finer, hazy, granular looking particles. At this stage the nucleus is very pale and the center very turbid, but the nuclear membrane still stains deeply and is intact. A few small chromatin granules are visible within the body of the nucleus. Toward the end the nucleus shrinks in size and retreats toward the periphery of the cell where it seems to be supported partly by the cell membrane and partly by clumps of debris; its center now is extremely turbid but the enclosing membrane still is very definitely outlined. Finally, the cell contents disappear completely and the cell membrane alone remains.

In most cases moderate bile imbibition is to be observed. It occurs in two forms: First, as very finely divided particles scattered over certain cells and, second, as aggregations of such particles lying in large vacuoles in the cytoplasm. The vascular and lymphatic channels lying between the rows of liver cells are usually greatly dilated, their endothelial lining being easily distinguished. The vascular channels contain a few pale red cells, shadow corpuscles, small numbers of leucocytes and debris. Sections stained in Sudan III reveal, in most cases, fat in small amount, appearing

mostly as rather large rounded masses, but occasionally as very minute particles lying in the cytoplasm.

The peculair material replacing the cytoplasm of the liver cells is insoluble in chloroform, ether, alcohol, equal parts of ether and alcohol, 1 per cent. sodium hydrate solution and 2 per cent. acetic acid solution. The last substance, however, renders it cloudy, so that the cell membrane is obscured. It is unaffected by prolonged exposure to 1 per cent. osmic acid solution, except to a very slight degree, a few small, blackish spots appearing here and there.

From these observations the following conclusions may be drawn:

1. Those pathological processes that appear to depend upon cytolysis present at least two definite types of structural changes in cells—the one characterized by rapid, simple solution of the hyaloplasm with preservation of the cell reticulum as, for example, in multiple non-inflammatory necrosis of the liver; the other by alteration of the hyaloplasm and reticulum with the formation of amorphous material, both the cell and nuclear membranes remaining very resistant, as in the liver cells just described. Extreme tenacity of the cell membrane appears to be the most constant single feature of all forms of cytolysis thus far recognized.

2. Polychromatophilia is associated with solution of the hæmoglobin of the red cells, especially in those conditions which are attended by destruction of blood. It appears not improbable that this peculiar reaction of the cell may represent a physiological as well as a pathological condition. As a physiological process it is conceivable that the polychromatophilia apparent in the earlier developmental stage of the red cells represents an attempt on the part of the organism to dispose of an excess of such cells by solution or that the cells have not yet received their full supply of hæmoglobin.

In closing I wish to express my appreciation of the interest shown in this work by Dr. Horst Oertel.

EXPERIMENTAL ATRESIA OF THE URETER.

By TORALD SOLLMANN, W. W. WILLIAMS AND C. E. BRIGGS.

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The effects of the complete and permanent occlusion of one ureter have been studied by numerous investigators. The older literature, which is summarized by Lindemann,[1] bears mainly on the morphological changes occurring in the kidney, the ureter of which was ligated. The more recent experiments[2] have dealt particularly with the changes observable in the opposite kidney, with the object of investigating the existence of auto-nephrotoxins. The functional changes produced in the kidney by ligation of its ureter have been touched upon only incidentally. This last condition suggested the present research, but in the course of this study we also controlled the morphological observations of previous investigators.

OPERATIONS AND MORPHOLOGICAL CHANGES.

Operations.—Four dogs were operated on August 7, 1903, and the right ureter ligated about 5 centimeters from the pelvis of the kidney. Dog (1) died after 31 days. Dog (2) sickened about the 72d day, and was killed on the 74th day. On the 107th day dogs (3) and (4) were re-operated under ether, and urinary fistulæ were established with the right kidneys. Dog (4) died 9 days later, or 116 days after the primary operation. Dog (3) was killed on the 185th day (the 78th day after the second operation).

The kidneys of all dogs showed more or less similar gross and microscopic changes with the exception of (4), in which the lesions were masked by a generalized renal infection.

Operated Kidneys.—The kidneys on the ligated side were enlarged and the pelvis contained from 88 to 250 c.c. of fluid. Minus

[1] Lindemann, *Zeitsch. f. klin. Med.*, 1898, xxxiv, 299.

[2] Pearce, *Univ. of Penna. Med. Bull.*, 1903, xvi, 217. Ames, *Jour. Path. and Bact.*, 1903, x, 265.

the fluid, the weight of the operated kidney was only one-fourth to two-thirds of that of the sound kidney. The capsule of the kidney as well as Bowman's capsule were slightly thickened. Most of the glomeruli were shrunken and many were replaced by fibrous tissue. The epithelial cells of the convoluted tubules showed cloudy swelling, necrosis and pressure atrophy. Most of the tubules were obliterated. The collecting tubules ran generally parallel to the cortex and showed slight dilatation in places. The interstitial tissue was increased, with slight round-cell infiltration. The arteries showed various degrees of endarteritis and periarteritis.

Sound Kidneys.—The kidneys on the non-ligated side were always slightly enlarged and microscopically showed slight cloudy swelling and moderate congestion especially of the medulla.

PROTOCOLLS.

Dog 1.—The right ureter was ligated 5 cm. from the kidney on August 7, 1903. The dog, without apparent cause, died October 7, 1903 (31 days).

Autopsy.—Putrefactive odor. Very small external scar. Internally small adhesions. Left kidney seems normal. Pelvis of the right kidney distended. Righ ureter dilated to about 1 cm. in diameter.

Sound kidney weighs	31 grams.
Operated kidney weighs	124 grams with liquid.
Operated kidney weighs	21 grams without liquid.

Therefore, 103 grams of liquid.

The right kidney consists of a sac with walls about 2 mm. in thickness.

Microscopic Examination.—Right kidney: The capsule is slightly thickened. The glomeruli show moderate thickening of Bowman's capsule. The capsular spaces are not dilated. Some tufts are considerably shrunken, with increased growth of fibrous tissue; and an occasional tuft is replaced by fibrous tissue. The convoluted tubules are for the most part obliterated, although there are occasional normal ones and others are lined with only a small fringe of ragged protoplasm containing apparently healthy nuclei. The collecting tubules are all pressed to one side and their general direction is parallel to the kidney capsule. Most of them have slightly flattened epithelial cells with nuclei in good condition. There is apparently slight dilatation of some. The blood vessels show moderate congestion. The arteries show slight internal thickening and more marked

periarteritis. The interstitial tissue is increased throughout, with a few small areas of round cell infiltration.

Left kidney shows slight cloudy swelling and moderate congestion especially of the medulla.

Dog 2.—The right ureter was ligated 5 cm. from the kidney on August 7, 1903. Reported sick on October 21. On October 23, dog receives morphin at 8:15 A. M. and ether at 8:45. Weight 10 kg. Cannula placed in left ureter at 9:24. Cannula placed in right ureter at 9:40. Urine flowed very slowly from the right kidney and ceased entirely at 10:15. At 10:30 A. M. 100 c.c. of 10 per cent. crystallized sodium sulphate was injected slowly into femoral vein. Dog died suddenly without assignable cause. Lived 74 days.

Sound kidney weighs	42 grams.
Operated kidney weighs	150 grams with liquid.
Operated kidney weighs	20 grams without liquid.

Therefore, 130 grams of liquid.

The pelvis of right kidney is markedly dilated.

Microscopic Examination.—Right kidney. The capsule is slightly thickened. The glomeruli show slight thickening of Bowman's capsule. Many capsular spaces are dilated and the tufts occupy one-third to one-half the space, the remaining portion filled with finely granular material. Some of the tufts are shrunken and many are obliterated. Nearly all of the convoluted tubules are obliterated or represented by a mass of nuclei surrounded by a little granular protoplasm. A few are considerably dilated and lined with large cuboidal epithelial cells. The collecting tubules run more or less parallel to the cortex. Many are obliterated and the majority of the remaining ones show varying degrees of dilatation. Many are filled with casts. The interstitial tissue is everywhere increased with small areas of leucocytic infiltration. There are a few small areas in the cortex that look like healed infarcts. The arteries show slight intimal and more marked adventitial thickening. Slight congestion. The pelvic lining is thickened and consists of several layers of rather large polygonal cells.

Left kidney as in dog No. 1.

Dog 3.—The right ureter was ligated 5 cm. from the kidney on August 7, 1903. On November 25 (107 days after the primary operation) a second operation was performed and 88 c.c. of urine were gently squeezed out and a urinary fistula was established. On the next day the dog was fairly lively. No food was given until November 30. On December 4 the skin was gaping, but the dog was lively. Ureter fistula was not patulous to a probe which could

be introduced only 5 cm. On February 11, 1904 (185 days after the primary operation and 78 days after the second) the dog was very well, although the abdominal wound was not quite healed. The fistula was moist. Could pass probe to kidney. 9:10 A. M. 2 c.c. 4 per cent. morphin, 9:30 ether. Right kidney found shrunken and empty. Cannulæ were placed in the ureters. The right remained perfectly dry; the left showed good secretion, as follows:

From 10:45 to 11:07 the average secretion of urine was 0.2 c.c. per minute.

From 11:07 to 11:10 225 c.c. of 5 per cent. cryst. sodium sulphate at 40° C. was injected into the femoral vein.

From 11:07 to 11:15 average secretion of urine was 1.75 c.c. per minute.

From 11:15 to 11:25 average secretion of urine was 3.6 c.c. per minute.

The right cannula remaining dry, it was reinserted nearer the kidney. It filled with a little bloody serum which could not have been more than 2 or 3 c.c. and no fluid was secreted during the experiment.

From 11:25 to 11:37 average secretion of urine was 2.6 per minute.

From 11:37 to 11:42 average secretion of urine from the left kidney almost ceased, being 0.2 c.c. per minute (probably due to shock).

From 11:42 to 11:44 second injection of 200 c.c. sodium sulphate solution. Left kidney starts secreting at once.

From 11:45 to 11:50 average secretion of urine was 2.5 per minute.

From 11:50 to 11:55 average secretion of urine was 2.5 per minute.

From 11:55 to 12:06 average secretion of urine was 2.5 per minute.

The left ureter was clamped at 12:08. Between 12:10 and 12:12 a third injection of 200 c.c. sodium sulphate solution was made. At 2 P. M. only 2 drops had escaped from the right ureter. The dog was killed.

Sound kidney weighs 43.2 gm.

Operated kidney weighs 9.6 gm.; about 4.5 mm. thick.

The right kidney is small, measuring 30 mm. in length, 16 mm. in breadth, and 8 mm. in thickness. On section it is very dense and the pelvis contains no fluid. The left kidney measures 41 mm. in length, 31 mm. in breadth, and 21 mm. in thickness.

Microscopic Examination.—Right kidney. The capsule is somewhat thickened. The glomeruli show slight thickening of Bowman's capsule. Many capsular spaces are dilated and the tufts are shrunken. A few glomeruli are obliterated and replaced by fibrous tissue. The convoluted tubules are mostly obliterated, some are represented by a confused mass of epithelial cells with their nuclei showing varying degrees of degeneration. In small discrete areas, usually near the cortex, are a few tubules lined with large epithelial cells and apparently in good condition. The collecting tubules are mostly obliterated and some of the remaining ones are dilated and contain casts. The interstitial tissue is increased throughout with a few small hæmorrhagic and scattered areas of leucocytic infiltration in it. The blood vessels are moderately congested and show marked adventitial thickening. The pelvic epithelium is thickened and consists of several layers of large cells.

Left kidney. The glomeruli are larger than normal; the tubules are apparently larger, with normal epithelial cells. Moderate congestion.

Dog 4.—The right ureter was ligated 5 cm. from kidney on August 7, 1903. On November 25 (107 days after the primary operation) a second operation was performed and 88 c.c. of urine were gently squeezed out and a urinary fistula established. On the next day the dog was fairly lively. No food was given until November 30. The dog gradually became sick and at noon of December 4 was comatose and died in the afternoon (116 days after the primary operation and 9 days after the second). The dog was kept frozen and was autopsied on December 7, 1903. No peritonitis. Right kidney was enormously enlarged with very thin walls and the pelvis was distended with 225 c.c. of reddish, turbid fluid, containing some pus. No sufficient cause of death could be assigned.

Microscopic Examination.—Right kidney. The capsule is much thickened and numerous vessels connect it with the cortex. Bowman's capsule is also considerably thickened. Most of the glomeruli are replaced by fibrous tissue, although a few tufts remain which are much shrunken and encroached upon by fibrous tissue. Practically no tubules are to be seen. The interstitial tissue is increased. The arteries show varying degrees of endarteritis and several rather large ones are thrombosed. There are a few small hæmorrhages. The greatest part of the sections is composed of necrotic tissue with here and there indefinite remains of glomeruli and tubules and everywhere marked infiltration with polymorphonuclear leucocytes predominating although no definite abscesses are to be seen.

CHEMICAL EXAMINATION OF THE FLUIDS IN THE OCCLUDED URETERS.

The results of these examinations are presented in Table I, the analytical methods appearing in the appended notes. Column III gives a summary of the fluid obtained after the first operation in the four dogs. The fluid from the left ureter of the congenital atresia reported by Dr. Allen[3] is also included in this table.

1. *Duration of the Atresia in the Human Case* (see page 82 of the JOURNAL).—In the thirteen years which elapsed since the establishment of the right kidney fistula, no urine was voided from the bladder, so that the left ureter must have been completely occluded for at least this period of time. It is probable, however, that the atresia was congenital, *i. e.*, that it persisted for 16 years.

2. *Quantity of Fluid.*—The quantities are only approximate, generally somewhat too low.

3. *Color of Human Fluid.*—After the precipitation of the proteid, the fluid has a light straw, serum color, which is slightly darkened by nitric acid and by heating with soda.

4. *Color of Dogs' Urine, 3 and 4.*—This is not altered by precipitating the proteids.

5. *Color of Second Accumulation of Fluid in Dog 4.*—This is fairly deep red, with an abundant slimy grayish red precipitate. The filtrate, after the removal of the proteids, is very faintly colored.

6. *Odor of Human Fluid.*—This is faint, somewhat like serum, on heating. It is not urinous, even on boiling with soda.

7. *Specific Gravity.*—This was taken with a picnometer in the human case, with areometers in the dogs.

8. *Depression of Freezing Point.*—Determined by Beckmann's apparatus, $\frac{\Delta}{\text{NaCl per cent.}} = 0.86$ in the human case.

9. *Total Solids.*—10 c.c. dried at 110° C.

10. *Ash.*—Incineration of 10 c.c. This is apt to show a trifle higher than it should, if the incineration is incomplete.

11. *NaCl.*—Incineration of 5 to 10 c.c. with $NaNO_3$ and Na_2CO_3, solution in HNO_3 to neutralization; titration with $AgNO_3$. Chromate indicator.

12. *Non-chloride Ash.*—Difference between total ash and NaCl. This is apt to be too high, see (10).

13. *SO_4.*—Gravimetrically with barium, in 20 c.c.

14. *P_2O_5.*—The determination of SO_4 and P_2O_5 is not very exact on account of the small quantity.

15. *Organic Solids.*—Difference between total solids and ash.

16. *Total Coagulable Proteid.*—10 to 50 c.c. of the fluid are acidulated with acetic acid and boiled with an equal volume of saturated sodium sulphate or chlorid. The precipitate is washed with water, alcohol and ether, dried at 110° C. and weighed.

[3] See page 82, this number of the JOURNAL.

TABLE I.

ANALYTICAL DATA OF THE FLUIDS OBTAINED FROM THE LIGATED KIDNEY.

Column I.	II.	III.	IV.	V.	VI.	VII.	VIII.
Experiment.	Human.	Summary of Dogs, 1 to 4. First Operation.	Dog 1.	Dog 2.	Dog 3.	Dog 4. First Operation.	Dog 4. Second Operation.
Time between occlusion of ureter and collection of fluid.	At least 13 years (1).	31 to 107 days.	31 days.	74 days.	107 days	107 days.	78 days.
Quantity of fluid (2).	200 c.c.	88 to 130 c.c.	103 c.c.	130 c.c.	88 c.c.	88 c.c.	225 c.c.
Color.	Faint amber, not urinous(3). Turbid.	Yellow urinous.	Fairly deep yellow.	Light yellow.	Quite deep yellow (4).	Deeper than with Dog 3.	Bloody, not urinous (5) Turbid.
Odor.	Faint, serum (6).	None.	Putrefactive.	None.	None.	None.	None.
Reaction to litmus.	Neutral.	All the dogs' urines have a slightly alkaline reaction.					
Sp. gr. (7).	1.0103	1.008 to 1.009	1.008	1.009	1.009	1.0085	1 018
Depression of freezing point	0.715°C (8)	—	—	—	—	—	—
Total solids % (9).	—	1.8 to 2.1	2.115	—	2.035	1.800	5.805
Ash % (10).	0.895	0.8 to 0.95	0.956	—	0.805	0.845	—
Cl, as NaCl % (11).	0.83	0.68 to 0.75	0.75	0 73	0.72	0 68	0.52
Non-chloride ash % (12).	0.065	0.085 to 0.206	0.206	—	0 085	0.165	—
SO_4 % (13)	—	0.021 to 0.036	—	—	0.021	0.036	—
P_2O_5 % (14).	0.046	0.033 to 0.065	—	—	0.033	0.065	—
Organic solids % (15).	—	0.955 to 1.230	1.159	—	1.230	0.955	—
Total Coagulable Proteid % (16).	0.25	0.390 to 0.858 Mean=0.74	0.858	0.657	0.820	0.390	3.932
Globulin(17).	Present.	Present.	—	—	Present.	0.175%	Present.
Albumin(18).	Present.	Present.	—	—	Present.	0.215%(19)	Present.
Non-coagulable Proteids (20).	Absent.	Probably absent in all the dogs' urines.					
Non-proteid Nitrogen % (21).	—	0.056 to 0.126 mean= 0.103	0.080	0.126	0.056	0.126	—
Urea % (22).	0.814 (24)	0? to 0.165 mean= 0.112	0.068	0.165 (25)	0?	0.156	—
Ammonia % (23).	Present.	0.0025 to 0.0054	0.0025	0.0054	—	—	—
Non-urea nitrogen % (26)	—	0.047 to 0.056	0.047	0.048	0.056	0.053	—
Reducing substance.	Small quantity.	—	—	—	—	—	—
Sediment.	Cellular, abundant.	—	Round cells of various types, largely degenerated.	A few blood and other cells (leucocytes?) largely degenerated.	—	—	Very abundant, many blood cells and considerable fibrin

17. *Globulin.*—Addition of equal volume of saturated ammonium sulphate.

18. *Albumin.*—Boiling the acidulated filtrate of (17). The Albumin : Globulin :: 1.23 : 1, in Dog 4.

19. The percentage of albumin is obtained by subtracting the globulin from the total proteid.

20. *Non-coagulable Proteid.*—Biuret test of filtrate of (16).

21. *Non-Proteid Nitrogen.*—Kjeldahl on filtrate of (16).

22. *Urea Nitrogen.*—Folin's method on filtrate of (16).

23. *Ammonia Nitrogen.*—Folin's method on filtrate of (16).

24. *Urea in Human Fluid.*—This was not determined by Folin's method, but by estimating the nitrogen in the boiled filtrate. This gave a small precipitate with phosphotungstic acid; some proteid had therefore probably escaped precipitation, and the figure for urea is too high, and unreliable. The presence of urea was shown qualitatively by the effervescence of a hypobromite solution.

25. *Qualitative Tests for Urea were Positive.*

26. *Non-Urea Nitrogen.*—Difference between (21) and urea nitrogen.

SUMMARY, DISCUSSIONS OF THE RESULTS, AND CONCLUSIONS.

Previous investigators have found that complete occlusion of the ureter may lead either to hydronephrosis or to atrophy. In Lindemann's series of six dogs, for instance:

Two animals showed simple hydronephrosis, three animals showed simple atrophy, and in one animal the kidney was slightly enlarged and the ureter and pelvis dilated, but fluid was absent. In his series of four rabbits, all showed hydronephrosis.

The result, whether hydronephrosis or atrophy, is evidently not determined by the time elapsing after the operation. Lindemann found that the intrapelvic pressure resulting from the ligation obliterates the lumen of the vessels, first of the veins and subsequently of the arteries; but that this is compensated by an increase of the collateral blood supply through the capsule, the degree of this compensation determining the presence or absence of hydronephrosis. If the blood supply is free, the fluid after tapping will accumulate again and again.

It is somewhat remarkable that all of our dogs showed hydronephrosis after the first operation. The results of establishing a urinary fistula differed in the two cases in which it was tried: The fluid did not re-form in Dog 3 even when sodium sulphate was

injected; whereas in Dog 4, a very abundant quantity of fluid re-accumulated spontaneously; but it differed notably in composition from the original fluid, having more the character of a purulent inflammatory exudate.

The histological changes consist in necrosis of the renal cells, obliteration of the glomeruli, increase of connective tissue, and endarteritis and periarteritis. Different areas in the same kidney are affected in very different degree, some areas appearing almost normal. The glomeruli are generally less altered than the tubules. The collecting tubules are generally displaced so as to run parallel to the surface; many are dilated. The changes correspond closely to those described by Lindemann.

The sound kidneys showed slight hyperæmia and hypertrophy, but no necrosis. This corresponds with the findings of Pearce and of Ames.

The uniformity in chemical composition of the fluid obtained, after the first operation, from the four dogs, as shown by Column III of Table I, is very striking, and points to a uniform origin by a process which is but little affected by the interval elapsing after the operation. The specific gravity, total solids and proteids correspond to those of a very dilute lymph, being but a trifle above those of cerebro-spinal fluid and aqueous humor, and much lower than those of serum, lymph and most cystic fluids (the proteid content of the latter being generally from 2 to 6.5 per cent.). The human fluid (Column II) which had remained in the kidney for a very long time had a particularly low proteid percentage; while that of the second fluid of Dog 4 (Column VIII) was very much higher; this last fluid having a pronounced inflammatory character and being of recent formation.

The absence of notable amounts of the specific urinary constituents is particularly important.[4] Odorous principles are entirely

[4] The research was originally undertaken at the suggestion of one of us (Briggs), to decide whether it would be possible to re-establish the function of the kidney after temporary ligation of the ureter. The answer must be in the negative, at least when a considerable interval elapses before the establishment of the fistula.

absent. Urinary pigments appear to be present in the four dogs' urines, but absent from the human case,[5] and after the second operation in case of the dogs. It seems fair to assume that the pigments were secreted shortly after the ligation, when the kidneys were still functional, and that they were reabsorbed with extreme slowness.

Urea was present in all the fluids, but its quantity was very small in the dogs, and probably in the human case. It is on the whole somewhat greater than in the serum (0.103 per cent., in place of 0.05 per cent.), but the difference may be within the analytical error. The same applies to the ammonia, phosphates and sulphates.

An important difference between these fluids on the one hand and blood serum, lymph and ordinary exudate on the other, lies in the higher contents of chlorid, and the consequently greater molecular concentration. The ordinary chlorid content of body fluid varies between 0.55 and 0.70 per cent., mean about 0.6 per cent (as NaCl), while that of the first kidney fluid, in the dogs, varied between 0.68 and 0.75 per cent., mean 0.725 per cent.; that of the human fluid was 0.83 per cent., that of the second fluid of Dog 4 only 0.52 per cent. The depression of the freezing point in the human case was 0.715° C., as against the normal value, for human serum, of 0.491 to 0.562. (Possibly the blood of this patient had a higher concentration than normal, since uræmia existed.)

The high chlorid percentage has evidently no relation to the length of time during which the fluid sojourned in the kidney. It is probably to be explained by the relatively slow absorption of this ion from the kidney pelvis. It is also to be remarked that cerebrospinal fluids generally have a somewhat high chlorid content (0.573 and 0.6 per cent.),[6] but this never reaches the height of these ureteral fluids.

Conclusion.—The fluid accumulating in the kidney, after unilateral ligation of the ureter, consists in the main of a transudate,

[5] Lindemann (*l. c.*) and Starling (Schaefer's Text-book of Physiology, 1898, i, 650) state that the fluid in atresia of the ureter is of light color, low specific gravity, and very low urea content.

[6] T. Sollmann, *Journ. Am. Med. Assoc.*, June 6, 1903.

poor in proteids, but somewhat enriched in chlorids and perhaps in urea, phosphates and sulphates. A small quantity of pigment is also retained. The fluid is probably formed by filtration through a filtering surface which is not freely permeable to proteids. A process of reabsorption also goes on simultaneously, in such a manner that the soluble solids are somewhat increased (perhaps to counterbalance the osmotic value of the serum proteids). There is no evidence that the remaining specific renal elements play any part in the formation of this fluid, although this part is not excluded.

We are indebted to Dr. Wm. T. Howard, Jr., for the preparation of the histological material and for valuable criticism.

HYDRONEPHROSIS OF RIGHT KIDNEY, CONGENITAL ATRESIA OF LEFT URETER, AND MARKED ATROPHY OF LEFT KIDNEY.

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The following case is sufficiently unusual to warrant its being recorded, and hence we desire to report it briefly.

In May, 1889, one of us (Allen) was called to see the patient, C. D., æt. 2½ years. The abdomen was markedly enlarged, the enlargement evidently being due to a collection of fluid filling the whole right side. An aspirating needle had been inserted on two different occasions, and the physician was positive that he had obtained the hooklets of an echinococcus. A diagnosis was therefore made of an echinococcus cyst of the liver and an operation was undertaken.

On May 4 the patient was anæsthetized and an incision parallel to the ribs was made upon the right side. A cyst was encountered from which a clear fluid was evacuated. This fluid resembled what is sometimes seen in a cyst of the echinococcus. It was preserved but no hooklets could be found in it. The amount of clear fluid removed was small, and it became at once apparent that there was another collection of fluid immediately below it. On carrying the incision more deeply, a cyst was opened containing a large amount of fluid, amber in color and having a urinary odor. After evacuation of the fluid, on insertion of the finger, the calices of the kidney could be distinctly palpated. It became evident, therefore, that the cyst was a greatly distended kidney. Finding this to be true an effort was made to extirpate it by shelling it out of the surrounding tissues. After it had been partially loosened, it was found, however, that the child's strength would not endure the completion of the operation, so that the portion of the cyst wall which had been set free, and which was made up of kidney tissue, was quickly removed, hæmorrhage being controlled by the application of an over-and-over

suture as the tissue was cut away. The cavity was then packed with gauze, a drainage tube being inserted outside of the gauze. The patient recovered from the operation and for a few weeks occasionally passed a few drachms of urine *per urethram*. After this, all the passage of urine by the natural channel ceased, and up to the time of the patient's death, February 3, 1902, he passed no urine whatever except through the opening in the side. The incision in the cyst wall gradually contracted, so that later a silver tube was fitted into it and to this was attached a rubber bag. The boy's growth was normal, he felt well and attended school. Aside from the leakage of urine around the tube in the side, which gave him a good deal of annoyance, and the fact that he could wear nothing to catch the urine at night, he seemed in normal health. This condition continued until June, 1900. At that time the tube began to give him considerable discomfort, the urine no longer flowed freely through it, but escaped around it. Consequently, on July 13, 1900, the patient was anæsthetized, the opening was somewhat enlarged and repacked. The patient was not seen again until the middle of January, 1902. At that time he came to the office looking extremely pale, and his mother said that he had suffered from severe hæmorrhage from the nose and there had also been a considerable discharge of blood from the side. In addition to the extreme pallor there was slight œdema of the face and legs. The heart action was greatly increased, and on examination it was found that the heart was much enlarged, the apex being displaced outward and downward. There was also a blowing, systolic murmur heard at the apex. The hæmorrhage from the kidney continued, and on February 3, 1902, the boy died, having developed marked symptoms of uræmia. An autopsy was made on the morning of February 4, conducted by one of us (Parker). The following is the report upon the case:

Anatomic Diagnosis.—Complete stenosis of right ureter at its renal end; hæmorrhagic pyelo-nephrosis with marked distention of right kidney; fistula communicating between the lower end of the right renal pelvis through the abdominal wall; hypertrophy of right kidney with acute and chronic interstitial nephritis and pressure atrophy; congenital atresia of left ureter; hydro-ureter; chronic

interstitial nephritis with marked atrophy of left kidney; slight dilatation of the right ureter associated with valve-like folds of the mucosa; hypertrophy of heart; congestion of lungs and spleen.

The protocol will be limited to the genito-urinary apparatus:

The external genitals are normal. The bladder is small, being 5 cm. long and 3 cm. in its greatest diameter. The right ureteral orifice is normal in position, and is readily found with the probe which passes into the ureter. The right ureter has two fusiform dilatations, the first is just below the pelvis and is 5 cm. long, the second is in the middle third and is 3 cm. long. The ureter is 18 cm. long, its greatest diameter is 12 mm., and its least diameter, 4 mm. The lumen is irregular, but is patulous from the bladder to within 1 cm. of the pelvis at the lower pole of the kidney, where it is obliterated by a fan shaped mass of fibrous tissue. On section, valve-like folds are found just below the fusiform dilatations and the mucosa is somewhat thickened. The ureter is in general empty, but here and there is a small amount of granular material on the mucosa. The right pelvis is markedly distended and is situated behind the kidney extending 3 cm. above its upper pole. It measures 12 cm. by 6 cm. by 4 cm., and is filled with fluid. At its lowest point the pelvis communicates with a fistulous tract which opens exteriorly in the right flank. On section, the pelvis is covered with a thin, grayish-yellow pseudo-membranous exudate, and the fluid is found to be hæmorrhagic with a urinous odor. The right kidney is embedded in fat and the lower pole is closely adherent to the abdominal parietes at the side of the fistula, causing it to be lifted forward and to the right. The exact measurement of the kidney is impossible, but it appears to be larger than normal and rests upon its own dilated pelvis. The other relations of the kidney are normal. The capsule is markedly adherent and the surface of the kidney is finely granular. Its consistence is somewhat increased. On section, it is pale and somewhat opaque and here and there is marked with small yellowish points. There are no large abscesses to be found. The kidney tissue is distorted and varies considerably in thickness, from 0.25 cm. to 2 cm. The thickest portion is the lower half of the anterior aspect, while on the posterior surface in places there is only a thin shell of tissue remaining. In most places the medulla suffers the most, though in others the cortex shares in the atrophy. The calices on the posterior surface are markedly distended, the anterior ones less so.

The left ureter is lobulated and has somewhat the appearance of a string of sausages. It is bow shaped and on its inner curvature measures 28 cm., on its outer, 34 cm. It is markedly dilated and varies from 3 to 4 cm. in diameter, and is nearly filled with about 200 c.c. of clear, amber-colored liquid which flows freely from one part to another. The ureter does not communicate with the bladder, although it extends to it. On section, the wall is thin, and valve-like folds are seen which cause narrowing of the lumen in places. The mucosa throughout is normal in appearance. The left pelvis is slightly enlarged and envelopes about two-thirds of the circumference of the kidney. It communicates only with a single calyx. The capsule of the left kidney is markedly adherent, but can be stripped off on one side. The surface is irregular and granular. A

few small cysts are scattered about. The consistency is markedly increased. The organ measures 2 cm. long, 8 mm. deep and 1 cm. thick. On section, it is very pale and very dense. The cortex is with difficulty differentiated from the medulla.

Histological Examination.—Right kidney. Sections from the thinner portions show rather distinct differences from those of the thicker parts. In the former, the glomeruli are either hyalin or fibroid. The tubules are few, shrunken, degenerating and show marked atrophy. Hyalin casts fill the lumina of some. There is a great increase of fibrous tissue with marked round cell infiltration. The arteries show thickening of the intima and many are obliterated. The pelvic lining shows marked fibrino-purulent inflammation, in which long slender bacteria are seen. In the thicker portion numerous glomeruli are increased in size, many of them being considerably larger than normal. Many, however, show fibrous thickening of the capsule with hyalin and fibrous changes in the tufts and so merge into the character of those described in the thinner parts. The tubules are apparently larger than normal, and the epithelial cells are generally swollen. In places numerous long, slender bacteria are seen in the lumina. In some places the interstitial tissue shows no change, while in others, there is marked increase with areas infiltrated with leucocytes. In these areas most of the leucocytes are polymorphonuclear neutrophiles, though there are many plasma cells and a few eosinophiles. Scattered throughout this portion of the kidney are larger and smaller abscesses, mostly in the medulla, causing necrosis of tissue with marked leucocytic infiltration. The arteries show no marked changes. There are a few small hæmorrhages. The pelvic lining shows an acute inflammatory process.

Left kidney. The sections consist chiefly of rather dense, fibrous tissue containing numerous nuclei. The cortex is very irregular. Apparently all the glomeruli are undergoing fibrosis, which is more or less marked, and many are replaced by a hyalin mass. The tubules are much reduced in number, and show various changes. A few are lined with a single row of cuboidal cells. Others are small and shrunken, with the lumina filled with granular detritus. Still others show varying degrees of dilatation up to cystic cavities which are from 50 to 100 mm. in diameter. Some of the tubules contain hyalin, and others colloid-like material. The interstitial tissue is everywhere greatly increased and in many places there is considerable round cell infiltration.

Cultures were made from the right kidney and bladder and from the right ureter. From the two former locations Bacillus coli communis was obtained; the culture from the right ureter remained sterile.

THE ELECTRICAL CHARGE OF TOXIN AND ANTITOXIN.

By CYRUS W. FIELD AND OSCAR TEAGUE.

(*From the Research Laboratory of the Department of Health, New York City.*)

Soon after the discovery of diphtheria antitoxin, several investigators attempted to convert diphtheria toxin into antitoxin by the electrical current, and some went so far as to suppose that this method would supersede the costly and time-consuming process of immunizing animals. Smirnow[1] inoculated rabbits with half a cubic centimeter of a two to three days old broth culture of diphtheria bacilli, and twenty-four hours later, when the animals were sick, injected 10 cubic centimeters of the anodal fluid, obtained by passing a current for eighteen hours through diphtheria toxin. According to him, the animals were saved by the injections. Bolton and Pease[2] stated that two cubic centimeters of the anodal fluid obtained from diphtheria toxin neutralized ten minimal lethal doses of the toxin. It is a well-established fact that acids destroy diphtheria toxin more readily than alkalies, and hence it is to be considered that it was the acid at anode which in Bolton and Pease's experiments neutralized the toxin. The latter investigators believed that the electric current caused a rearrangement of the constituent atoms of the toxin molecule, so that antitoxin resulted; but they did not determine whether the toxin molecule moved with or against the current by virtue of the charge which it carried.

The first to undertake the determination of the electro-positive or electro-negative nature of diphtheria toxin and antitoxin was Römer.[3] Römer used a U-shaped tube and allowed the electrodes to dip into the toxin and antitoxin to be investigated. After the current had been passed for a stated interval of time, the fluid was pipetted from both branches of the tube simultaneously and tested

[1] Smirnow, *Berl. klin. Woch.*, 1892, xxxii, 645.

[2] Bolton and Pease, *Jour. of Exper. Med.*, 1896, i, 537.

[3] Romer, *Berl. klin. Woch.*, 1904, xli, 209.

on guinea-pigs for toxic or antitoxic properties. The results were entirely negative, since he was unable to determine whether the toxin or antitoxin particles traveled toward the cathode or anode. This failure was due primarily, we believe, to the destruction of the toxin and antitoxin by the products of electrolysis, brought about by the strong current employed, for Römer states that he used oil to get rid of the bubbles at the electrodes. Furthermore, the method of simultaneous pipetting very likely caused some mixing of the different portions of the fluid.

To avoid these errors in technique and to eliminate the effects of electrolytic products, we first used a modification of Bilitzer's cells, as shown in Diagram 1. The cells and connecting tubes were of

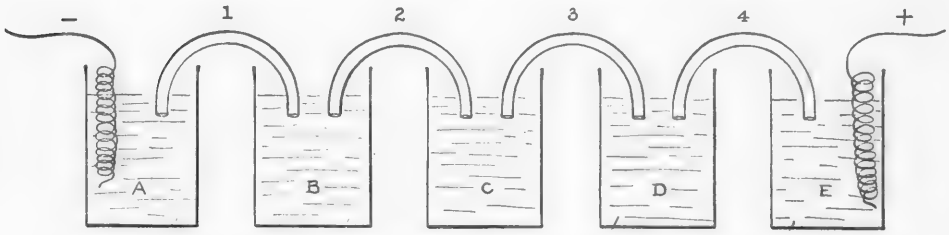

Fig. 1.

glass, the electrodes consisted of coils of platinum wire. The cells *A, B, D* and *E* were filled with distilled water, which was brought to the same level in all of them by means of water-filled connecting tubes. The central cell, *C,* was then filled to a slightly lower level than the others with the toxin or antitoxin to be tested. The small connecting tubes *1* and *4* being in place, and the direct lighting current turned on, the tubes *2* and *3* were filled with distilled water and simultaneously placed in position, thus completing the circuit. After the current had been passed for the desired length of time, the connecting tubes *2* and *3* were removed at the same moment, care being taken that they remained filled. Then *1* and *4* were removed. Under the influence of the current, the fluid in *A* became alkaline, that in *E,* acid, while *B* and *D* remained almost neutral. Hence, only the fluid in the two latter cells was tested on guinea-pigs for toxic or antitoxic value. The results

were unsatisfactory and contradictory, probably owing to the fact that some of the test fluid passed into the neighboring cells along the outside surface of the connecting tubes, being drawn up by capillarity. When we used longer connecting tubes, the internal resistance became too great for the passage of an appreciable amount of current. This method was therefore discarded.

The following apparatus yielded decisive results. Two semicircular glass tubes, each 1 cm. in diameter and 20 cm. long, were filled with melted agar (2 per cent. agar in distilled water) and allowed to cool to the temperature of the room. These were then arranged as in Diagram 2, the same platinum electrodes being used as in the previous experiments. The toxin or antitoxin to be tested was placed in the middle beaker, distilled water, in the other two.

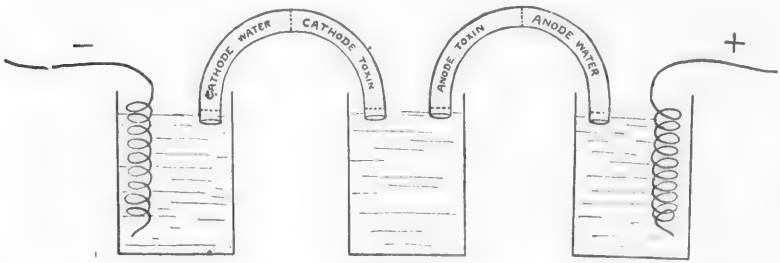

FIG. 2.

The direct Edison street lighting current, 110 volts, was passed for from four to five hours,[4] the distilled water in the end beakers being siphoned off and renewed every half hour to eliminate the disturbing influence of the products of electrolysis. The agar tubes were now removed and the ends thoroughly rinsed in distilled water. The agar mass was then forced from that portion of the tube which dipped into the toxin or antitoxin out through the other end. About a quarter of an inch was removed from each end of the mass, and the remainder divided approximately in half. Each

[4] The current was passed for only four or five hours because the risk of a disturbance due to products of electrolysis increased with the time; moreover, Bredig, Hardy, Pauli, and others found that the passage of a current for twenty-four hours or more would often cause a reversal of the charge carried by particles which would then necessarily be driven back in the opposite direction, thus obscuring the nature of the charge which they originally carried.

of these portions was rinsed in distilled water, chopped into fine pieces, and allowed to stand for one hour in about 6 c.c. of distilled water. At the end of this time the agar was removed by filtering through gauze, and the filtrate was tested on guinea-pigs for toxic or antitoxic properties. The accompanying table gives the results of these experiments.

TABLE I.

Substance Tested by Passage of Electric Current.	Acidity or Alkalinity.	Cathode Water.	Cathode Toxin or Antitoxin.	Anode Toxin or Antitoxin.	Anode Water.
Diphtheria toxin.	Acid.	No reaction.	+ in 20 hours.	No reaction.	No reaction.
	Alkaline.	No reaction.	+ in 28 hours.	No reaction.	No reaction.
Tetanus toxin.	Acid.	No reaction.	+ in 48 hours.	No reaction.	No reaction.
	Alkaline.	No reaction.	+ in 40 hours.	No reaction.	No reaction.
Diphtheria antitoxic serum. Tested against 35 M. L. D.'s.	Acid.	Protected.	Protected.	+ in 22 hours.	+ in 24 hours.
	Alkaline.	Protected.	Protected.	+ in 42 hours.	+ in 38 hours.
Diphtheria antitoxic globulins.[5] Tested against 35 M. L. D.'s.	Acid.	+ in 36 hours.	Protected.	+ in 36 hours.	+ in 36 hours.
	Alkaline.	+ in 24 hours.	Protected.	+ in 36 hours.	+ in 36 hours.
Tetanus antitoxic serum. Tested against 35 M. L. D.'s.	Acid.	Protected.	Protected.	+ in 50 hours.	+ in 68 hours.
	Alkaline.	Protected.	Protected.	+ in 48 hours.	+ in 48 hours.
Normal broth. No toxin.	Acid.	No reaction.	No reaction.	No reaction.	No reaction.
	Alkaline.	No reaction.	No reaction.	No reaction.	No reaction.
Normal horse serum. No antitoxin. Tested against 35 M. L. D.'s.	Acid.	+ in 36 hours.	+ in 36 hours.	+ in 36 hours.	+ in 36 hours.
	Alkaline.	+ in 40 hours.	+ in 42 hours.	+ in 40 hours.	+ in 40 hours.
Test without the electric current. Diphtheria toxin. Diphtheria antitoxin.[5] Tested against 35 M. L. D.'s.		Water half negative + in 40 hours.		Toxin half negative + in 41 hours.	

The first .5 cm. of the agar dipping into the toxin or antitoxin was removed, as was always done in our experiments with the electric current. There was always a slight trace of toxin or antitoxin in the first half centimeter, but it was never found beyond this point. A similar phenomenon was observed by Flexner and Noguchi, in relation to the diffusion of tetanus toxin into agar.

[5] Gibson, *Jour. of Biol. Chem.*, 1905, i, 161; Flexner and Noguchi, *Jour. of Exper. Med.*, 1906, viii, 547.

In order that the table may be more readily understood, a detailed explanation of one of the experiments (No. 6, for example) contained in it may not be superfluous.

Diphtheria antitoxic serum was made slightly alkaline to phenolphthalein by the addition of a small amount of 0.1 normal sodium hydrate. The agar tubes were placed in position, as in Diagram 2, and the current was passed through it for four hours. The four portions of agar, which we shall designate as cathode water, cathode-antitoxin, anode water, and anode-antitoxin, were then cut into fine pieces and extracted in water for one hour, and to each of these extracts was added 35 m. l. d. of diphtheria toxin. These four portions of fluid were now injected subcutaneously into the adbominal walls of four guinea-pigs each weighing about 250 grams.

The two guinea-pigs receiving the fluid from the anode water and anode-antitoxin portions of agar died in thirty-eight and forty-two hours, respectively; hence, these portions must have contained no antitoxin. Those receiving the fluid from the cathode water and cathode-antitoxin portions showed no induration at the point of injection and no loss of weight for five days, after which they were discharged. Hence, they were fully protected against the 35 m. l. d. of toxin.

The table shows that under the influence of an electric current, the particles of both toxin and antitoxin travel toward the cathode, and that a change in the reaction of the solvent does not cause a reversal of the charge carried by the particles.

Blitz, Much and Siebert[6] claim that both tetanus toxin and tetanus antitoxin are precipitated by electro-positive inorganic colloids and conclude, therefore, that they are electro-negative and should, under the influence of an electric current, pass toward the anode. However, neither they nor Römer were able to demonstrate this fact experimentally. It is probable that the precipitates which they obtained were due either to the action of electrolytes contained in the toxin or antitoxin on their inorganic colloids, or to the action of these colloids on non-toxic or non-antitoxic protein substances, or to a combination of both. It is worthy of note that their non-

[6] Blitz, Much and Siebert, *Beit. zu exper. Therapie,* 1905.

toxic broth gave precipitates with practically the same inorganic colloids as did the toxin.

Hardy[7] states that proteins are amphoteric; that is, that in an acid medium they travel toward the cathode and in an alkaline medium toward the anode, while in a neutral medium they do not move toward either pole. He worked with an albumen coagulated by heat. Pauli,[8] using a protein solution obtained by dialyzing serum from eight to ten weeks and filtering off the euglobulin, found, in agreement with Hardy, that the protein was electro-positive in an acid solution and electro-negative in an alkaline one.[9] We have shown that alteration of the reaction of the solvent does not change the character of the charge carried by particles of toxin or antitoxin. If proteins are amphoteric, as is generally believed, then this observation of ours would point to the non-protein nature of toxin and antitoxin. In one series of experiments, however, we have found that the portion of agar containing the toxin or antitoxin, that is, the cathode portion, was the only one to give the biuret reaction and this occurred when the test substance was in either an acid or an alkaline solution. If experiments which we have under way should verify the last results, it would indicate that native proteins may not be amphoteric and then the argument that we have advanced as to the non-protein nature of toxin and antitoxin would be invalidated.[10]

If the combination of toxin with antitoxin is a true chemical reaction, one would expect that under the influence of an electric current toxin would travel in one direction and antitoxin in the opposite direction. Such, however, was not found to be the case and we are, therefore, inclined to believe that this union is not a true chemical reaction, but a matter of adsorption, as was first suggested by Bordet, and has since been claimed by others.

[7] Hardy, *Jour. of Physiology*, 1899, xxiv, 288.

[8] Pauli, *Hofmeister's Beit.*, 1906, vii, 531.

[9] Pauli denaturalized his proteids by the prolonged dialysis.

[10] Oppenheimer (Toxin and Antitoxin, 1903) in summing up the work on this subject concluded that toxin and antitoxins are of non-protein nature. Quite recently Osborn, Mendel and Harris (*Amer. Jour. Physiol.*, 1905, xiv, 259), working with ricin, have taken issue with him, having found that their purest product still gave protein reactions. As a matter of fact, nothing definite is known at present of the chemical nature of these various substances.

CONCLUSIONS.

1. Both diphtheria and tetanus toxin and their antitoxins are electro-positive, that is, they pass to the cathode under the influence of an electric current.

2. The character of the charge is not altered by a change in the reaction of the solvent.

3. The combination of toxin and antitoxin would seem to represent not a true chemical reaction but the adsorption of one colloid by another.

AN EXPERIMENTAL STUDY INTO THE CAUSE OF THE INCREASED PORTAL PRESSURE IN PORTAL CIRRHOSIS.[1]

By FREDERICK C. HERRICK, Cleveland.

I.

In reviewing the subject of portal cirrhosis, it is evident that our understanding of the cause, course and pathological physiology of the disease is far from clear. The pathological anatomy is fortunately well understood and one need but to refer to the recent compilation by Dr. Rolleston to find a clear description and statistics of every phase of the process. But when we enter the domain of the pathological physiology of the disease not one of its chief departures from the normal physiology is satisfactorily explained.

The increase in the portal blood pressure is one of the most marked abnormal conditions developed. As evidence that this condition exists we have the frequent hæmorrhages, the collateral circulation and the ascites, by many considered as a result of increased portal pressure. The fact of this increase in pressure cannot be doubted, but the cause of this condition, heretofore considered to be the obstruction to the portal vessels by the fibrosis and its contraction, is, in the light of the following observations, open to question.

The object of this research was to explain the increased portal pressure in this disease and thus to throw further light on the collateral circulations frequently developed naturally as well as following the operation of Talma and similar operations. In order to gain this end the circulation of the normal liver was first studied.

A short historical resume may not be out of place.

[1] The following experimental work was done in the Pathological Laboratory of the London Hospital, London, England. I am greatly indebted to Dr. W. C. Miller, pathologist to the hospital, for this privilege and also for the material which was necessary for the work.

Betz[2] in 1863 worked in Ludwig's laboratory on the question: Does the amount of blood entering the liver through the two vessels depend on their relative pressures? If this is so he recognized that the arterial flow if of large enough volume would exclude the portal. He injected many livers of dogs with hardening fluid and measured the corresponding arteries and veins, finding their proportion in size to be as 1:5 though there were wide variations. Large arterial trunks were frequently near relatively small portal vessels. The relative volume flow between the hepatic artery and the portal vein he found to be as 1: from 2 to 4 when the two vessels were flowing at different times. When flowing during the same time, they were as 1:50. His arterial pressure was twice the portal which is of course much below the normal ratio (1: from 13 to 15). Still this shows the influence of the portal pressure in decreasing the arterial flow.

Gad[3] in 1873 wrote a dissertation based upon experimental work in which he concluded that the arterial circulation in the liver performed a double function in that it brought oxygen and mechanically controlled the portal flow. A further point suggested by Gad was the aspirating action of the arterial on the portal current as the former passed by the openings of the latter, joining with them at an acute angle. He further found by experiments with rabbit's livers that a current through the arteries retarded the portal flow.

To this observation may be added the fact that the hepatic artery is capable of bringing the pre-urea bodies to the liver for their formation into urea and even in health this occurs to a certain extent.[4] At least four cases are on record in which the portal vein was entirely obliterated and the individuals lived for years; two were congenital malformations.[5] Dogs with Eck fistulas and with their portals tied excrete a normal amount of urea and have been kept for months in good health. It is therefore certain that the arterial circulation has for its functions (1) the nourishment of the connective tissue of the liver, (2) an influence on the amount of blood passing through the portal vessels, this influence being a factor in the circulatory balance within the liver, (3) the power to carry sufficient blood to the liver lobule to ensure a normal excretion of urea and an activity of the liver function sufficient to maintain health.

II.

Within the normal liver there are six factors to be considered.

1. A large volume-circulation with a low pressure.

2. A small volume-circulation with a high pressure.

3. A common channel of exit for fluid entering by these two circulations.

[2] Betz, *Zeit. für rat. Med.*, 1863, xviii, 44.

[3] Gad, Dissertation, Berlin, 1873.

[4] Hahn, Massen, Nencki, Pawlow, *Arch. f. exper. Path. und Pharm.*, 1893, xxxii, 161. Herrick, *Jour. of Exper. Med.*, 1905, vii, 751.

[5] Abernethy, *Philosophical Trans.*, 793, p. 61; Kierman, *Philosophical Trans.*, 1833, p. 758; Lawrence, *Medico-Chirurg. Trans.*, 1814, v, 174; Osler, Practice of Med., 1905.

4. A freely expansible tissue framework.

5. Two methods by which the entering circulations may influence each other, *i. e.*, by direct communication or by lateral pressure.

6. A vaso-motor mechanism to both sets of vessels.

Considering the large volume-circulation, Quincke (5) quotes von Basch who estimated the portal pressure when the splanchnic nerve was cut in a dog, at from 7 to 16 mm. of mercury, and Heidenhain who found it from 5.2 to 7.2 mm. without nerve section. The volume-flow of the portal vein was estimated by Cybulski (5) in a "small dog" at from 2.4 to 2.7 c.c. per second (a proportionate estimate in a man of 70 kilos would give the portal flow as about 35 c.c. per second). I have not been able to find a record of estimates of the volume-flow of the hepatic artery or its blood pressure. So far as size is concerned it may be considered as at least equal to the brachial artery, which has a pressure of 130 mm.

The freely expansible tissue framework is a most important factor towards the maintaining of the proper balance between the two circulations. This point and the next one will be discussed later when the experimental data have been given. Schäfer[6] concludes that both the intrahepatic arterial and portal vessels possess vaso-motor constricting powers. His evidence is not entirely convincing. A study of this influence, however, has not been included in this research, the aim being to draw some conclusions regarding the capacities of the intrahepatic veins and arteries, considering them as so many anastomosing, more or less expansive tubules lying adjacent to each other in a framework of varying expansibility.

That the widely differing pressures of the portal vein and hepatic artery at the porta hepatis come to a common level at the junction of their inter-lobular or intra-lobular venules is certain. This equalization comes about through the medium of the following factors:

1. A direct communication exists between the veins returning from the arterial supply and the portal venules. Regarding this I quote from Landois.[7] "The branches of the hepatic artery

[6] Schäfer, Text-book of Physiology, 1900, 140, 643.

[7] Landois, Text-book of Physiology. p. 308.

throughout their entire course accompany the larger branches of the portal vein (to which as well as to the adjacent larger bile ducts they supply nutrient capillaries). These branches enter into numerous anastomotic communications among themselves. The small capillaries pass mainly from the periphery of the acinus into the capillaries, however, that lie in the thicker connective tissue upon the larger venous and biliary branches and pass over chiefly into two venous trunks that, accompanying the corresponding arterial branches for some distance, empty into branches of the portal vein. Individual arterial branches pass up to the surface of the liver, where they form a wide nutritive network, particularly under the peritoneal covering. The small venous radicles collecting from this point also reach the ramifications of the portal vein."

The arterial supply thus passes through a capillary system arranged in Glisson's capsule about the portal interlobular veins and its pressure becomes reduced to an ordinary venous one. This arterial division has also been described as resulting in the formation of three or four arterioles which wind spirally about their accompanying portal vein. This spiral arrangement can be demonstrated in microscopical sections.

2. The vaso-motor influence in balancing these flows must be considered. Thus it has been shown by Bayliss and Starling that a stimulation of the splanchnic nerves causes an increased flow of blood from the liver, although the portal vein was tied. It has also been shown by Mall[8] that the portal vein receives fibers from the splanchnics. In my work in making the Eck fistula, I have frequently noticed slow vermicular contractions of the portal vein following mechanical stimulation of its walls. Two Italian authors ascribe both constrictor and dilator fibers to the splanchnic nerves and dilator fibers to the vagus (Cavazzani and Manca[9]).

Thus, in the normal liver through the medium of these two factors the arterial and portal pressures reach a common level at the points where their currents join to form the intralobular veins. If the arterial supply to a given area is relatively increased a disturbance of the balance will occur. Also, a fibrosis will make the vessel

[8] Mall, *Arch. f. Physiol.*, 1892, 409 (from Schäfer).

[9] Cavazzani and Manca, *Arch. ital. de biol.*, 1895, xxiv, 35, 295.

walls more rigid, less variable in caliber and if this intra-hepatic balance is partly due to a vaso-motor influence, this must be altered by an advancing fibrosis. I therefore sought to determine the influence on each other as to pressure and volume-flow of changes in the portal and arterial pressures and to measure the volume-flow of the two currents entering the liver at pressures considered as normal. I next made the same observations upon cirrhotic livers and found a marked difference, as will be shown.

III.

The methods employed were as follows:

For normal livers only those were used which were free from possible disease. The history and pathological diagnosis will be given. No livers altered by passive congestion were used; only typical cirrhotic livers were used for that part of the research which concerns this condition. Microscopical sections were made of each liver and the condition verified. The livers were taken never longer than twenty-four hours post mortem and were never kept longer than twenty-four hours after being removed from the body. When they were kept over night it was always in normal saline in cold storage. Comparisons of the two circulations in the *same* liver only were made. The two circulations in different livers were not compared. The circulating fluids used were normal saline solution at 38–39° C. At the end of the experiments I used defibrinated sheep's blood fresh from the animals for verification. The livers were always immersed in their natural position in normal saline at body temperature. Mercury manometers were used to measure pressures. In every case the liver was washed of blood at the normal pressures of 130 mm. for the arterial and 10 mm. for the portal vessels. They were next washed backward by a pressure of 10 to 20 mm. through the hepatic veins. It required from ten to fifteen minutes to wash a liver preparatory to the experiments. Bits of washed fibrin were occasionally floated out and especially backward from the hepatic to the portal vein. The first estimations were always made on both circulations together at normal pressures. These were then varied within the limits of from zero to 140 mm. arterial, and zero to 20 mm. portal pressure. The pressures were always put on together, and variations made as nearly as possible in the normal ratio. The portal circulation was next studied alone at variable pressures. The arterial was then considered alone with its higher pressures. By this order an unnatural dilatation of the vessels was prevented.

After the experiments the livers were sliced into sections one centimeter thick and the macroscopic vessels examined for possible occluding fibrin. In but one case was fibrin found in a portal vessel of importance, a vein of about three millimeters diameter. Sections for histological study were then made.

IV.

The effects on the portal pressure of changes in the arterial pressure and vice versa were first studied.

Arterial Pressure.	Portal Pressure, Normal Liver.	Portal Pressure, Cirrhotic Liver.
0	10	10
30	10	15
50	10	18
100	11	20
130	12	30
250	14	35

Portal Pressure.	Arterial Pressure, Normal Liver.	Arterial Pressure, Cirrhotic Liver.
5	130	130
30	130	135
40	135	140
10	127 (?)	133

In normal livers an increase in the arterial pressure, when the portal was flowing at normal pressure of 10 mm., caused a very slight rise in the portal pressure, *i. e.*, about 1 mm. rise of portal for every 40 mm. rise of arterial pressure. In making this observation the portal was first set at 10 mm. and, when flowing evenly, the arterial flow was gradually turned on. Thus, raising the arterial pressure from zero to 130 mm. caused a rise of but 3 or 4 mm. in the portal pressure. When this same observation was made on a liver with marked portal cirrhosis a great difference was noted. In this case a rise of 130 mm. in the arterial pressure caused a rise of from 20 to 30 mm. in the portal pressure.

With the normal liver the rise of *portal* pressure was 1 mm. for every 40 mm. of arterial pressure increase; with the cirrhotic liver it was 1 mm. for every 6 mm. of arterial pressure increase.

When in a normal liver the *arterial* pressure was first set at 130 mm. and the portal gradually opened a similar result was noted. The arterial rise was 1 mm. for every 7 mm. increase in portal pressure.

A similar observation in a cirrhotic liver was obviously impossible, for so soon as the arterial pressure was set at 130 mm. the portal pressure rose immediately to from 20 to 25 mm., although the portal flow had not been opened, and this occurred, though more slowly, regardless of whether the portal vessels were *full*

or not. If they had been drained *before* this observation the fluid flowed into them, as will be shown in the next observation, filled them and registered the pressure.

After this arterial pressure was thus set and the portal pressure was stationary at say 25 mm., a further rise in portal pressure, caused by turning on its flow, was followed by a rise in arterial pressure of 1 mm. for every 3 mm. increase in portal pressure. Thus in the normal liver the *arterial* pressure rose 1 mm. for every 7 mm. increase in portal pressure; in the cirrhotic liver it rose 1 mm. for every 3 mm. increase in portal pressure.

It will be seen that arterial rise in pressure did not affect the portal pressure in normal livers until it had passed 100 mm. I did not measure the effects of pressures above 150 mm. In cirrhotic livers the effect on the portal pressure was evident at 30 mm. arterial pressure. These effects of one pressure on the other occurred immediately on changing a pressure and show as clearly as possible the mutual influence between the portal and arterial pressures within the liver, and is, I believe, an important explanation of the rise in portal pressure in portal cirrhosis.

As an explanation of this marked effect on each other in portal cirrhosis I may offer the following suggestions:

1. The arterial supply to the fibrous tissue of a cirrhotic liver is increased. Thus an increased amount of fluid passes through the arterial capillaries, enters the portal venules and raises the portal pressure there.

2. This would be especially the case if the hepatic veins were not quite able to give passage to the increased volume of fluid. (During life a slight weakening of the heart muscle might favor this backward pressure.)

3. There was a freer flow of fluid through the arterial capillaries into the portal veins, as will be shown later. This could occur by a dilation of these capillaries which would favor a greater influence of the arterial on the portal pressure.

V.

The arterial and portal pressures in the normal liver are perfectly balanced at the point where the arterial return veins enter

the portal venules. In the liver of portal cirrhosis this balance is disturbed by (1) the larger arterial supply, (2) the loss of portal influence by lateral pressure on the arterial capillaries and the freer communication of the arterial with the portal vessels by means of dilated capillaries. This free anastomosis between the arterial and portal vessels within the liver has been shown above (Landois). As further evidence of these communications I wish to cite the following observation:

The arterial flow alone was connected, leaving the portal and hepatic veins with a glass cannula tied in each for collecting the fluid returning through them. There was always a free return flow through both veins from an arterial inflow. Further, in the normal liver, the portal return flow was less in volume than that from the hepatic vein. In the markedly portal cirrhotic liver the portal return flow was always greater than that from the hepatic vein. This is what we would expect from the previous observations on pressure.

When the inflow was through the *portal* vein and the hepatic vein and artery were left open the return flow was *entirely* through the hepatic vein in both normal and cirrhotic livers. No fluid returned through the hepatic artery. This observation may be explained in one of the following ways:

1. Gad described the arterial return venules as uniting with the portal at an acute angle leaving a wedge-shaped valve between them. With a like pressure in the portal veins and these entering venules there would be a free flow through both; but if the pressure of either was increased there would be a narrowing of the other's lumen and reduction in its flow. If there is no arterial flow it is evident the large portal volume will entirely occlude the entering veins from the arterial capillaries, and fluid cannot flow back through them, as seen above. When, on the other hand, there is no portal flow and there is a flow through the arteries, their channels being of much less volume than the portal veins, are manifestly unable to occlude the portal vessels and hence the fluid returns through them.

2. The fact that no fluid returned through the hepatic artery from a portal inflow may also be explained by the intralobular capil-

laries and hepatic vein offering less resistance than the arterial capillaries. Against this, however, is the fact that in the cirrhotic livers where the arterial capillaries are dilated no fluid returned through them and the hepatic artery.

3. Any obstruction in the hepatic veins might cause this regurgitation of fluid through the portals. I cannot find any evidence of such obstruction. There was no intra-lobular fibrosis in these livers and, from the experimental side, there were no occluding blood clots. We must, therefore, conclude that this is not an explanation of the above facts. The wedge-shaped valves of Gad, together with the difficulty of fluids regurgitating through a capillary network, must explain the fact that an inflow through the portal returns entirely through the hepatic veins.

VI.

The volume of flow from the portal and arterial vessels is the subject of the next observations to which I wish to draw attention. Certain proportions were evident, as shown in the following table. The time during which each flow was measured was one minute.

	Volume flow of portal vein.				Vol. flow of portal vein in cirrhosis calculated proportionately to weight of normal livers.
		10 mm.	20 mm.	30 mm.	
Normal	45 oz.	230 c.c.	313 c.c.		
	52 oz.	422 c.c.	662 c.c.		
Cirrhotic	68 oz.	758 c.c.	1144 c.c		10 mm. 20 mm.
	128 oz.	880 c.c.	1230 c.c.	2100 c.c.	668 c.c. 996 c.c.
					Actual vol. flow.
					819 c.c. 1187 c.c.

From this table it will be seen that the average volume flow of the portal vein in the cirrhotic livers was, in the proportion to their weights, greater than that of the normal livers. In a normal liver weighing 45 oz. the flow from the portal vein alone was 230 c.c. in one minute at 10 mm. pressure; in another weighing 52 oz. the flow was 422 c.c. at the same pressure.

In a cirrhotic liver weighing 68 oz. (portal cirrhosis) with roughened surface and adherent thickened capsule the portal volume flow in one minute at 10 mm. pressure was 758 c.c. In another weighing 128 oz. a typical large "hob nailed" liver with marked peri-portal fibrosis, opaque thickened capsule, many adhesions, the

portal vessel allowed a flow of 880 c.c. in one minute at 10 mm. pressure. Thus the portal vascular capacity in portal cirrhosis, so far from being *decreased,* appears to be proportionately *increased.* These comparisons were verified on other livers as well and it was found that from a cirrhotic liver flowed at the same pressure at least as much fluid proportionately to its weight as from the normal one. This shows that there can be no obstruction to the portal vessels within the liver from fibrous tissue. The portal vessels allowed fluid to pass freely and it was evident as soon as such a liver was transfused that the flow was quite as free as that through a normal liver.

So soon as the arterial flow was added a different state of affairs existed. Now the portal flow was decidedly limited.

With a normal liver weighing 61 oz.

Art. Pres.	Port. Pres.	Time.	Vol.
130 mm.	10 mm.	1 min.	1024 c.c. Art.
			632 c.c. Port.
		Repeated.	1032 c.c. Art.
			650 c.c. Port.
130 mm.	20 mm.	1 min.	1100 c.c. Art.
			1300 c.c. Port.
		Repeated.	1060 c.c. Art.
			1280 c.c. Port.

With a cirrhotic liver weighing 128 oz.

Art. Pres.	Port. Pres.	Time.	Vol.
130 mm.	25 mm.	1 min.	1250 c.c. Art.
			1050 c.c. Port.
130 mm.	30 mm.	1 min.	1750 c.c. Art.
			1250 c.c. Port.
130 mm.	40 mm.	1 min.	1550 c.c. Art.
			2750 c.c. Port.
130 mm.	50 mm.	1 min.	1525 c.c. Art.
			2950 c.c. Port.

Thus at 130 mm. arterial and 10 mm. portal pressure the arterial volume-flow was the larger. It is generally conceded that the portal volume-flow is normally greater. This relation occurred between 10 and 20 mm. portal pressure. In the cirrhotic liver this relation occurred only at a portal pressure between 30 and 40 mm. Up to this point the arterial flow was decidedly preponderant.

In the above observations on a cirrhotic liver the portal pressures are seen to be very high. As shown in Part IV, when such a liver was placed under observation it was found that the influence of one circulation on the other was much more marked than in a normal organ. With an arterial pressure of 130 mm. in the hepatic artery it was found impossible to have a normal portal pressure of 10 mm. The portal pressure immediately went to 20 mm., although the portal flow *had not been opened.* In making the above observations, therefore, the arterial pressure was first set at 130 mm., and after it was flowing evenly and the pressure in the manometer connected with the portal vein had risen to its maximum, the portal flow was opened and regulated so as to add from 5 to 30 mm. more pressure.

In a normal liver, to overcome the arterial resistance to the portal flow the portal pressure had to be raised above 10 mm. At 10 mm. portal pressure and 130 mm. arterial pressure the arterial flow was greater. When the portal pressure was 20 mm. its flow became greater.

In a cirrhotic liver the portal pressure necessary to resist 130 mm. arterial pressure was much higher, being 40 mm. The circulatory balance is maintained in a cirrhotic liver at a portal pressure much above that considered as normal. By circulatory balance I have meant the pressures at which the arterial and portal vessels, when flowing together, allowed the flow of the largest amounts of fluid.

Conclusions.

1. In the liver of portal cirrhosis there is a far freer communication between the arterial and portal currents than in the normal liver.

2. Factors contributing to the increased portal pressure in portal cirrhosis are (1) the direct communication of the arterial pressure to the portal vessels through dilated capillaries, (2) the larger volume-flow of the hepatic artery in proportion to the portal flow in cirrhosis as compared to that in the normal liver.

3. A portal cirrhotic liver gives passage to an amount of portal fluid proportionate to its weight. There is no obstruction to the portal vessels from fibrosis in the large portal cirrhotic liver.

4. From an arterial inflow there is a free return flow through the portal as well as through the hepatic veins in both normal and cirrhotic livers.

5. From a portal inflow the return is through the hepatic vein only. The Gad's theory of valves and the arterial capillary network account for this fact.

6. The portal pressure has a decided influence on the arterial volume-flow and vice versa. This influence is more marked in the cirrhotic than in the normal liver.

7. The communication of the arterial pressure to the portal pressure is an important factor in an explanation of the increased portal pressure in portal cirrhosis.

AUTOPSY 959. (Year 1905.) Female, 18 yrs., burned on thorax, head, arms; death eighteen hours later. Liver normal, wt. 52 oz. Histological examination showed normal liver tissue.

AUTOPSY 953. Female, 21 yrs. Perforating gastric ulcer; death twenty hours later from general peritonitis. Liver, normal, wt. 45 oz. Microscopical examination showed a normal liver tissue.

AUTOPSY 970. Male, 48 yrs., brought in dead. Post mortem diagnosis: fibrosis of mycardium and partial occlusion of the coronary arteries by atheroma. Liver, 61 oz. Capsule thin, normal. Microscopical examination showed normal liver tissue.

AUTOPSY 998. Female, 60 yrs., general arterio-sclerosis, bronchitis, emphysema; arterio-sclerotic kidneys. Liver wt. 68 oz., surface rough and irregular, diaphragm adherent, capsule thickened, substance of liver brown, fibrous, tough. Microscope showed peri-portal fibrosis.

AUTOPSY 1055. Male, 44 yrs. Cause of death, injury. Liver, large, tough, pale. Microscopical examination shows rather a marked peri-portal fibrosis, in places isolating the lobules from each other.

AUTOPSY 1086. Male, 63 yrs. Epithelioma of penis. Liver wt. 128 oz., hob nailed, tawny yellow, tough, capsule opaque and thickened. Many adhesions about porta hepatis, and between the capsule and the diaphragm. Microscopical examination showed a typical, peri-portal fibrosis of marked degree.